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U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY—Bulletin No. 52, Part I.
D. E. SALMON, D. V. M., Chief of Bureau.

EXPERIMENTS CONCERNING TUBERCULOSIS,

UNDER THE SUPERVISION OF

THE BIOCHEMIC DIVISION.

PART I.

The Virulence of Human and Bovine Tubercle Bacilli for Guinea Pigs and Rabbits.

BY

MARION DORSET, M. D.,
Assistant Chief of Biochemic Division, Bureau of Animal Industry.

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(Continued on third page of cover.)

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[a] Transferred August 16, 1902, to the U. S. Public Health and Marine Hospital Service, as Chief of Division of Zoology, but remains also in temporary charge of Zoological Division, Bureau of Animal Industry.

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BUREAU OF ANIMAL INDUSTRY—Bulletin No. 52, Part I.
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WASHINGTON:
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1904.

LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY,
Washington, D. C., November 24, 1903.

SIR: I have the honor to transmit herewith a manuscript entitled "The virulence of human and bovine tubercle bacilli for guinea pigs and rabbits," by Dr. Marion Dorset, assistant chief of the Biochemic Division of this Bureau. This manuscript forms the first part of a bulletin on the general subject of "Experiments concerning tuberculosis," and I recommend therefore that this paper, with the two others to follow, be published as Bulletin No. 52 of this Bureau. The illustrations, consisting of two half-tone and three colored plates, are necessary to a clear exposition of the text of Part I.

Respectfully,

D. E. SALMON, *Chief.*

Hon. JAMES WILSON, *Secretary.*

LETTER OF SUBMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry, Biochemic Division,
Washington, D. C., November 13, 1903.

Sir: I herewith submit for publication Part I of Bulletin No. 52 on "Experiments concerning tuberculosis."

These experiments have been made for the purpose of studying the virulence of tuberculosis bacilli obtained from man and other animals. The work upon the large animals has been done by the Biochemic Division in conjunction with the Experiment Station of the Bureau. The studies with the small experimental animals and the general morphology have been made in the Biochemic Division.

Abstracts of the results of some of this work have been printed in various medical journals, but to secure the complete publication, with illustrations, it has seemed advisable to issue a bulletin in parts.

Part I refers to the comparative virulence of human and bovine tubercle bacilli for guinea pigs and rabbits, by Dr. M. Dorset.

Respectfully,

E. A. de Schweinitz,
Chief of Biochemic Division.

Dr. D. E. Salmon,
Chief of Bureau.

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EXPERIMENTS CONCERNING TUBERCULOSIS.

INTRODUCTION.

By E. A. de Schweinitz, Ph. D., M. D.
Chief of Biochemic Division.

Though the difference in virulence of tuberculosis bacilli from various sources had been recognized for a long time, it was not until the publication of Koch's paper, which was read in London at the Tuberculosis Congress in 1901, that careful, systematic investigations in regard to the possibility of the transmissibility of bovine tuberculosis to man were extensively made. To determine this point positively it would be necessary to experiment upon man with bovine tuberculous material. As such experiments are not permissible, the other ways which have been followed in this study are the following:

(1) The comparative study of the virulence of human and bovine tubercle bacilli for small experimental animals.

(2) A study of the comparative virulence of human tubercle bacilli for pigs and cattle. These experiments include tests upon cattle with human tubercle bacilli obtained from the various organs and secretions of both adults and children.

(3) A study of the comparative virulence of turbercle bacilli of human and bovine origin for those animals most closely allied to man, namely, monkeys.

(4) A study of the morphological changes in both human and bovine tubercle bacilli produced by continued growth on various sorts of culture media, the influence of such media on the virulence or disease-producing power of these various bacilli, and the influence on the morphology and virulence of tubercle bacilli produced by their development in the animal body or upon various sorts of artificial media.

(5) A study of the cases of tuberculosis on record where the infection can apparently be traced to bovine tuberculous material.

(6) A chemical examination of the composition of tubercle bacilli obtained from various sources, and an examination of the different substances secreted and excreted by these bacilli grown upon artificial media.

(7) A comparison of the tuberculins made from cultures of tuberculosis of various animals.

(8) Experiments to immunize cattle against tuberculosis by previously inoculating these animals with attenuated human bacilli.

While a summary of the results of our own experiments and those of others along similar lines will be published in Part III of this bulletin, the following general conclusions drawn from our experiments may be given:

It is possible to obtain from cases of human tuberculosis germs which will produce tuberculosis in pigs, cattle, and monkeys upon subcutaneous inoculation. While it is possible by means of a number of different tests to draw fair conclusions as to the probable origin of these virulent tubercle bacilli, from a practical standpoint this is not of great importance. If we obtain from man a tuberculosis germ which will kill cattle on subcutaneous inoculation, and believe this germ to be originally of bovine origin, then it follows that bovine bacilli may infect man. If the tests show that the bacillus is of human origin, then the intertransmissibility of bovine and human tuberculosis is proved.

The genetic relation of tubercle bacilli of various origins has apparently been well established. While there is a great difference in virulence of various bacilli from different or similar sources, it is universally admitted that bovine bacilli are the most virulent for all species of animals upon which they have been tried; and the conclusions, apart from positive cases and experiments, that the bovine bacilli are virulent for other animals, including man, is perfectly natural and justifiable.

The experiments recorded in the several parts of this bulletin will substantiate the brief résumé given.

Part I.—THE VIRULENCE OF HUMAN AND BOVINE TUBERCLE BACILLI FOR GUINEA PIGS AND RABBITS.

By M. Dorset, M. D.,
Assistant Chief of Biochemic Division.

The experiments which are here recorded in Part I of this bulletin deal solely with the virulence of human and bovine tubercle bacilli for guinea pigs and rabbits, together with some observations upon the morphological and cultural characteristics of bacilli from both sources, the question of the intertransmissibility of human and bovine tuberculosis being only indirectly considered. The terms "bovine bacilli" and "human bacilli" are used in this paper to indicate the source from which the various organisms were originally obtained.

REVIEW OF THE LITERATURE.

The question as to the morphological and cultural identity of all mammalian tubercle bacilli was raised by Smith,[1][a] who also directed attention to the comparative differences in virulence of human and bovine tubercle bacilli for guinea pigs and rabbits.

In the researches just referred to, Smith carried out a most painstaking and exhaustive study of seven cultures from human sputum, six cultures from tuberculous cattle, one culture from a hog, one from a cat, and one from a case of tuberculosis in a horse. The cultures were all made on hardened dog's serum, from the organs of guinea pigs which had been inoculated with the sputum or with tissue from the above-mentioned animals. After a large number of comparative examinations, he formulates the following conclusions as to the morphology and cultural characteristics of human and bovine bacilli:

> (1) Bovine and other animal bacilli (except Nasua, which is regarded as coming from man) grew less vigorously for a number of generations than the sputum bacilli. Sputum I is an exception and is probably an atypical form.
>
> (2) Bovine bacilli are much less influenced by certain modifications of the culture medium.
>
> (3) Bovine bacilli tend to remain short; human bacilli are either more slender from the start or become so during cultivation.

In testing the relative virulence of human and bovine tubercle bacilli for guinea pigs, Smith found that after intraperitoneal inoculation the disease progressed so rapidly that no difference in the virulence of the two varieties could be noticed. When, however, guinea pigs weighing from 400 to 700 grams were inoculated subcutaneously with 0.5 c. c. of a suspension of tubercle bacilli in bouillon, the suspension having a density about equal to a 24-hour typhoid culture, the differences were more marked. The bovine guinea pigs died in from 40 to 51 days, while 2 "sputum" guinea pigs were chloroformed 77 and 100 days, respectively, after inoculation, the third animal having died on the forty-fourth day. The livers and lungs of the "bovine" guinea pigs and the 3 "human" guinea pigs were examined histologically. The distinguishing features were the much more extensive involvement of tissue and the much larger number of tubercle bacilli present in the lesions of the "bovine" animals. In the lungs the only distinctive feature was the great abundance of tubercle bacilli in the case of the "bovine" guinea pigs.

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[a] Superior figures refer to the bibliography at the end of the article.

The author sums up the differences observed after the inoculation of guinea pigs with human and bovine cultures as follows: "The more rapid death of all guinea pigs inoculated with bovine bacilli, the greater extent of the lesions and the far greater abundance of tubercle bacilli in them."

For his experiments upon rabbits Doctor Smith used animals weighing from 1,300 to 2,200 grams. The usual dose of culture was 0.5 c. c., administered intravenously. The differences noted in rabbits of this size are described by the author in a summary, as follows:

> (1) Death of the bovine cases in 17 to 21 days.
>
> (2) Rapid evolution and necrosis of the pulmonary tubercles, with very great increase of the tubercle bacilli in them.
>
> (3) Death of the "sputum" rabbits (one explainable exception) did not ensue, but after 1½ to 3½ months the original weight had been more or less exceeded.
>
> (4) The pulmonary tubercles in the "sputum" rabbit developed very slowly and with very little tendency toward necrosis. The bacilli were present in very small numbers only.
>
> (5) The swine and cat cultures are to be classed with the bovine; the horse culture stands intermediate.

The author continued his work further to experiments upon mice, pigeons, and cattle, but inasmuch as those investigations have no direct bearing upon the experiments which I wish to describe, that work will not be reviewed in this paper.

In 1901 Ravenel[2] published the results of his studies upon the comparative virulence of human and bovine tubercle bacilli. He studied the morphology and cultural characteristics of seven human and six bovine cultures and tested their pathogenic power for guinea pigs, rabbits, horses, pigs, sheep, dogs, cats, and calves. His conclusions are as follows:

> (1) The tubercle bacillus from bovine sources has in cultures fairly constant and persistent peculiarities of growth and morphology by which it may be tentatively differentiated from that ordinarily found in man.

(2) That cultures from the two sources differ markedly in pathogenic power, affording further means of differentiation, the bovine bacillus being very much more active than the human for all species of animals tested with the possible exception of swine, which are highly susceptible to both.

The author, however, notes one exception in the case of his "culture F" (bovine). This organism, which had previously grown very scantily, began in the eighth generation to grow luxuriantly, and at the same time its morphology so changed that it could have passed for a typical human culture. Its pathogenic power for guinea pigs and rabbits was not so great as is usually found in bovine cultures.

In a comparison of bovine cultures H and L and human cultures K and M, the guinea pigs inoculated subcutaneously with the two bovine cultures lived an average of 28½ and 26⅔ days, respectively, while the animals inoculated with the two human cultures lived an average of 38 and 34 days, respectively. His rabbit inoculations were all made subcutaneously and so could not form a satisfactory comparison with the work of Smith nor with my own, as all of the rabbit inoculations which will be described later were made intravenously.

In a later communication[3] Ravenel describes two cultures of exalted virulence obtained from the mesenteric glands of tuberculous children. One of the cultures (B B) corresponded morphologically and culturally more nearly to the bovine type (as previously defined by Smith and himself) than to the human.

Karlinski[4] states that he found no cultural differences whatever between human and bovine tuberculosis bacilli, except in the rapidity of their growth.

The work of Vagedes[5] is very interesting in this connection, although it was not undertaken for the purpose of comparing human and bovine tubercle bacilli but rather to study the variations in mammalian bacilli, the question of the nonidentity of human and bovine bacilli not being considered. The author isolated twenty-eight cultures of tubercle bacilli from various forms of human tuberculosis and two cultures from nodules in the lungs of tuberculous cattle. His cultures were made on solidified blood serum, to which 2.5 per cent glycerine was added, and were for the most part isolated directly from the diseased tissues without a preliminary passage through guinea pigs. Weighed quantities of the cultures were brought to a state of fine subdivision and suspended in normal salt solution before inoculation.

Guinea pigs were found to be so susceptible to all of the cultures that no differences in pathogenic power for them could be observed. When, however, rabbits were inoculated intravenously, differences became apparent. His rabbits weighed from 1,650 to 1,950 grams. As a result of these rabbit inoculations, he was able to separate his cultures into three classes. Class I included those which in a dose of from one-third to one-fourth milligram produced a generalized tuberculosis in the course of 1 or 2 months. In this class were four human cultures and the two bovine cultures. The second class of cultures was of medium virulence, requiring a larger dose to produce a generalized tuberculosis, while the third class included those which even as the result of a large dose showed extensive changes in the lungs only, the disease not becoming generalized. The subcutaneous inoculations of rabbits showed human culture W and bovine culture I to be the most virulent of all, producing a generalized tuberculosis within a month.

The culture medium used seemed to have little influence upon the virulence of the various cultures, and twelve successive passages of a moderately virulent culture through a rabbit did not noticeably affect its virulence.

Very little difference in the character of the growths on culture media was observed by the author. The bovine cultures I and II and the above-mentioned virulent human culture W were remarkable on account of the strikingly short form of the individual bacilli, none of them exceeding 1.5 μ in length. Especial attention is drawn to the fact that the cultures showing the shortest form in culture media were also the most virulent for rabbits. The author also states that those organisms possessing the greatest virulence for animals were isolated from especially malignant cases of tuberculosis in man.

Lartigau[6] has reported briefly a series of experiments which were apparently similar to those of Vagedes, although the details were not given. He isolated nineteen cultures of the tuberculosis bacillus from a variety of human tuberculous lesions and found a wide variation in their virulence for rabbits and guinea pigs. One of his most virulent cultures grew very scantily and slowly, and on account of these properties and its morphological characters the author considers it as closely resembling the bovine bacillus. His other virulent cultures did not conform to this standard, however, as is shown by the following statement: "No relation could be observed between the morphology and virulence of the tubercle bacilli investigated."

Among other conclusions drawn from the work were the following: "Tubercle bacilli of great virulence were on the whole more difficult of cultivation, and the growths scantier and shorter lived than were tubercle bacilli of less virulence. Tubercle bacilli of widely different virulence may be present in any one of the various human tuberculosis lesions."

Lartigau also says that tubercle bacilli of feeble virulence are to be expected in those cases of pulmonary tuberculosis where the disease is progressing very slowly, thus agreeing with Vagedes, as noted above.

In some recently published experiments Veszprémi[7] records the following results:

By the intravenous injection of various human tubercle bacilli into rabbits it was possible to separate the cultures into several classes, the separation being based upon their virulence. Bacilli of very weak virulence produced a considerable number of tubercles; these did not become caseous, but usually showed retrogressive changes. Bacilli of medium virulence produced a large number of tubercles, but no evidence of a toxic effect was noted. Bacilli of great virulence produced tubercles in which the bacilli multiplied and brought about marked and widespread caseation, and they furthermore exhibited a strongly toxic action. They also appeared to possess the power of disseminating themselves rapidly and overwhelming the animal. The author found by comparing the clinical history of the cases from which his cultures were isolated with the results which he obtained by the inoculation of rabbits that the most virulent cultures came from those cases which were either of recent origin or which had run a rapid course. Owing to the comparatively small number of cultures which he isolated, he was not able to form positive conclusions, but his experiments may be considered as evidence indicating that it is from the most rapidly fatal cases of tuberculosis that we may expect to derive the most virulent cultures of the tubercle bacilli. In these conclusions he agrees with Vagedes and Lartigau.

Krompecher & Zimmermann[8] record the results of their investigations concerning the virulence of thirty cultures of the tubercle bacillus derived from as many cases of surgical tuberculosis in human beings. These authors lay great stress upon the fact that all of their cultures were made upon glycerinized potato directly from the human tissues, and they are of the opinion that all systematic studies of the comparative virulence of tubercle bacilli found in various cases of human tuberculosis are unreliable if the virus has been passed through an animal previous to making the pure cultures, and such bacilli, in their opinion, should be classed as animal and not human bacilli. Without going into an extended discussion of this question, it is believed that such an assumption on the part of Krompecher & Zimmermann is unjustifiable in the light of my own experiments and those of other observers. Smith[1] obtained all of his cultures by a preliminary passage through a guinea pig, and he records no increase in the virulence of the feebler cultures and no decrease in that of the more potent ones. Vagedes[5] records the successive passage of a culture of medium virulence through twelve rabbits without any noticeable change in its virulence. I have also had occasion to pass a human culture of moderate virulence and a bovine culture each successively through six rabbits without any apparent modification of their virulence.

Krompecher & Zimmermann also express the opinion that all tubercle bacilli from cases of surgical tuberculosis and a part at least of those from pulmonary tuberculosis are of about equal virulence, and they intimate that those cultures from pulmonary cases which possess an exalted virulence may owe their greater power to a mixed infection. The different course of the various tuberculous diseases is, in their opinion, due rather to a difference in the disposition of the various tissues and organs than to differences in virulence between the infecting bacilli. In regard to this latter statement, I am of the opinion that both the virulence of the bacilli and the individual susceptibility of the infected persons must be taken into account in explaining the variations in the course of different cases of tuberculosis.

Strauss[9] mentions the very considerable resistance shown by rabbits to tuberculosis, and calls attention to the fact that, although the spleen and liver may present tuberculous lesions after subcutaneous inoculation, they are not pronounced, the lungs and kidneys being the organs which are most extensively diseased. He probably refers to human tuberculous virus, although the source of his culture and the details of work are not given.

Prudden & Hodenpyl[10] were the first to describe the effect produced upon rabbits by the intravenous injection of dead tubercle bacilli. They do not state the source of their cultures, though it was presumably human. The chief lesions produced by their inoculations were many small nodules in the lungs and liver—a few in the spleen. These lesions showed no tendency whatever to a cheesy degeneration, and the longer the animal was allowed to live the fewer were the tubercle bacilli that could be found in the lesions. Later investigators have shown[11] that a certain amount of caseation may result from the injection of dead tubercle bacilli, although such a process must necessarily be limited, as the bacilli can not multiply.

A brief summary of the literature just quoted shows that Smith found all of his bovine cultures more virulent for guinea pigs and rabbits than his human cultures; Ravenel finds the same, with two exceptions: One bovine culture after short cultivation approached the human type and one human culture, virulent for calves upon intravenous inoculation, corresponded more nearly to the bovine than the human type in its morphological and cultural features. Its virulence for rabbits and guinea pigs was not reported. The work of Vagedes and Lartigau shows that there may be a wide variation in the virulence and morphology of tubercle bacilli of human origin, and the former describes a human bacillus which was equal in pathogenic power to bovine cultures which he also had under cultivation.

It can be seen that, although Smith in his first report noted differences which were most marked between human and bovine bacilli in their effect upon guinea pigs and especially rabbits, no attempt has been made to confirm his results except by Ravenel.

It was, therefore, with the idea that the inoculation of rabbits might perhaps prove to be an easy and simple method of distinguishing between human and bovine tubercle bacilli that the experiments now to be described were undertaken. It is a well-known fact that tubercle bacilli are very tenacious of their virulence, and if it can be shown that all tuberculous virus of bovine origin possesses an exalted virulence for rabbits, as is indicated by Smith's work, and that all tubercle bacilli from cases of human tuberculosis are lacking in this property, the rabbit test would be a most efficient aid in determining the source of the contagion in those cases of human disease where there is reason to suspect infection from bovine sources.

I have followed as closely as possible the methods detailed by Smith[1] in the Journal of Experimental Medicine, except in the matter of a culture medium, as will be explained later.

THE EXPERIMENTS IN DETAIL.

THE CULTURE MEDIUM.

For the isolation and propagation of all cultures, a medium consisting of the mixed white and yolk of hen's eggs was used. This medium has been previously described,[12] but owing to the fact that some slight changes have been made in the method of preparation, it seems best to review the process as it is now carried out.

The egg shell is broken carefully and the entire contents dropped into a wide-mouthed sterile flask. The yolk may be broken with a sterile platinum wire. The flask should be shaken until the white and yolk are thoroughly mixed, and 25 c. c. of distilled water added to every four eggs. The mixture is then strained through a sterile cloth. This procedure serves to remove the foam produced by shaking, and in addition furnishes a homogeneous medium which will not otherwise be obtained. The egg is now poured into sterile tubes, care being taken to avoid foaming, and the tubes, each containing about 10 c. c. of the medium, are inclined in a blood serum oven and hardened at a temperature of 73° to 76° C. This hardening usually covers 3 days—4 or 5 hours each day. During the first 2 days the temperature is maintained at 73° C., and on the last day it is carried to 76° C. This prolonged intermittent heating, in addition to producing a firm medium, almost always accomplishes a perfect sterilization. A small dish of water is kept in the oven during the entire time of heating in order to prevent drying. After sterilization and hardening have been completed we have a firm, opaque, yellowish medium, which usually contains very little water of condensation. Since moisture, as shown by Smith, is very necessary to a satisfactory development of the tubercle bacillus, it is often advisable to add a few drops of sterile distilled water to the egg tubes just before inoculation. If the water be added before hardening the surface of the medium is apt to be too soft.

In making cultures from tissues, a small piece of the material is well mashed with a pair of forceps, transferred with a platinum wire to the

DESCRIPTION OF PLATE I.

FIG. 1.—Twenty-eight days' culture of human tubercle bacillus, E T, on solidified dog's serum, fifth generation. Preparation stained with cold carbol-fuchsin and decolorized with 10 per cent sulphuric acid as described in text. Magnified 1,200 diameters. Photograph by James A. Emery.

FIG. 2.—Twenty-eight days' culture of bovine tubercle bacillus, C No. 81, on solidified dog's serum, fifth generation. Preparation stained with cold carbol-fuchsin and decolorized with 10 per cent sulphuric acid as described in text. Magnified 1,200 diameters. Photograph by James A. Emery.

DESCRIPTION OF PLATE II.

FIG. 1.—Twenty-eight days' culture of human tubercle bacillus, C No. 4, on solidified dog's serum, fifth generation. Preparation stained with cold carbol-fuchsin and decolorized with 10 per cent sulphuric acid as described in text. Magnified 1,200 diameters. Photograph by James A. Emery.

FIG. 2.—Twenty-eight days' culture of human tubercle bacillus, S E No. 3, on solidified dog's serum, fifth generation. Preparation stained with cold carbol-fuchsin and decolorized with 10 per cent sulphuric acid as described in text. Magnified 1,200 diameters. Photograph by James A. Emery.

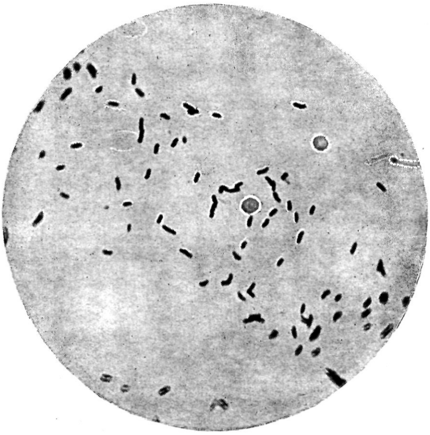

Fig. 1.

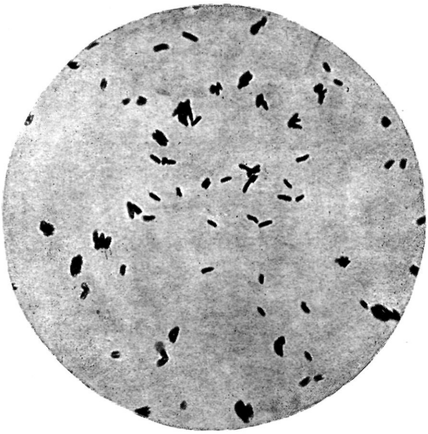

Fig. 2.

TUBERCULOSIS BACILLI, GROWN ON DOG'S SERUM.

Fig. 1, human bacilli; fig. 2, bovine bacilli.

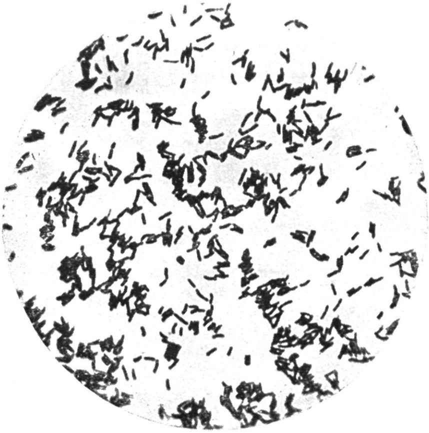

Fig. 1.

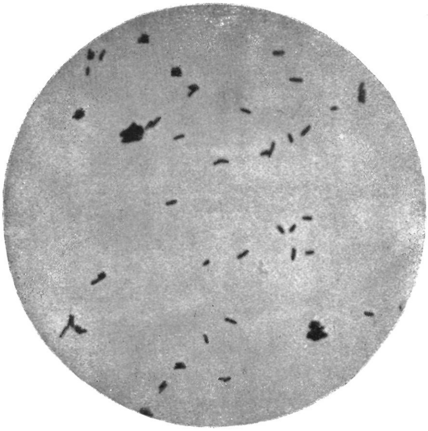

Fig. 2.

TUBERCULOSIS BACILLI, GROWN ON DOG'S SERUM

Figs. 1 and 2, human bacilli.

surface of the egg and rubbed well over the surface, the bit of tissue being allowed to remain in the tube. The cotton plug is cut off even with the mouth of the tube and pushed in about a sixteenth of an inch. Paraffin is now poured on the plug until it is completely sealed. The tubes are placed in the incubator at 38° C. in an inclined position. Growth of the tubercle bacillus is usually plainly visible in from 2 to 3 weeks, and I have frequently seen it much sooner. Cover-glass preparations have shown a distinct increase in the number of bacilli in 3 to 5 days. When the growth becomes visible it is usually in the form of minute round colonies dotted over the surface of the egg.

THE CULTURES.

The cultures used in this work were all obtained from guinea pigs in the manner just described, and all were freshly isolated with the exception of human No. 50 and bovine III. The guinea pigs were inoculated subcutaneously with bits of tissue from the various cases of tuberculosis, except in the case of the two animals from which the sputum cultures were obtained. The latter were inoculated intraperitoneally.

(1) *Culture F T, human.*—Obtained from the lung of a guinea pig which had been inoculated with sputum from a 45-year-old woman suffering from pulmonary tuberculosis.

(2) *Culture F L, human.*—Obtained from the spleen of a guinea pig which had been inoculated with sputum from a 40-year-old man suffering from pulmonary tuberculosis.

(3) *Culture S E No. 3.*—Obtained from the spleen of a guinea pig inoculated with a small fragment of the lung of a 50-year-old man who had died of generalized tuberculosis.

(4) *Culture G D, human.*—Obtained from the spleen of a guinea pig which had been inoculated with a small fragment of a peritoneal tubercle from a 19-year-old man who had died of a generalized tuberculosis.

(5) *Culture 12124, human.*—Obtained from the spleen of a guinea pig which had been inoculated with a small fragment of the lung of a 27-year-old woman who had died of tuberculosis, the autopsy showing extensive tuberculous disease of the lungs and tuberculous ulceration of the intestines, with swelling of the mesenteric glands.

(6) *Culture C No. 1, human.*—Obtained from the spleen of a guinea pig which had been inoculated with a small fragment of a mesenteric gland from a 19-months-old child that had died of a generalized miliary tuberculosis, together with extensive ulceration of the large and small intestines.

(7) *Culture D T, human.*—Obtained from the spleen of a guinea pig which had been inoculated with a small fragment of the lung of a child that had died of a generalized tuberculosis.

(8) *Culture E T, human.*—Obtained from the spleen of a guinea pig which was inoculated with a small fragment of a mesenteric gland from a 12-year-old girl who had died of an intestinal and peritoneal tuberculosis.

(9) *Culture C No. 4, human.*—Obtained from the spleen of a guinea pig which was inoculated with a small fragment of a peritoneal tubercle from a child of 5 years that had died of a generalized tuberculosis.

(10) *Culture No. 50, human.*—Obtained from the spleen of a guinea pig which had been inoculated with a bit of spleen from a tuberculous baboon. The baboon was inoculated with a human culture which had been under cultivation in this laboratory for a long time, a history of the culture not being available.

(11) *Culture C No. 81, bovine.*—Obtained from the spleen of a guinea pig which had been inoculated with a fragment of a mediastinal gland from a cow suffering from tuberculosis; natural infection.

(12) *Culture III, bovine.*—Obtained from the spleen of a guinea pig which had been inoculated with a small fragment of the lung of a tuberculous baboon. The baboon had been inoculated with a small amount of bovine III (Smith) tubercle culture. This culture had previously been grown for some time on bouillon in addition to having been cultivated on blood serum for a number of generations before reaching our hands.

It can be seen from the above record that a considerable variety of human cultures was used, all, with one exception, being recently isolated. Only one freshly isolated bovine culture was used. That culture, however, was fully equal in virulence to any of the bovine cultures described by Smith, and was considered entirely satisfactory as a standard with which to compare the human cultures.

CULTURAL CHARACTERS.

Macroscopic appearance of cultures.—All examinations as to the morphological and cultural peculiarities were made upon cultures which were of the same age and which had been grown, under identical conditions, upon the same lot of culture media.

Egg cultures.—In examining cultures upon the egg medium one feature of the growths was quite constant in the case of all the different strains of tubercle bacilli. This was the appearance of small, round, distinct colonies over the inoculated surface, the number of such colonies depending, of course, upon the number of bacilli in the tissue which is introduced. Transfers from one tube to another never exhibited the colony formation, owing to the relatively large number of organisms introduced.

A close examination of the gross characters of the various cultures, both originals and subcultures, made it possible to separate them into two groups. These differences were slight, and it must be borne in mind that a more prolonged cultivation may entirely obliterate the dividing line.

Group I (consisting of cultures E T and C No. 4, human; C 81, and III, bovine) was characterized by the very scanty growth, by the fact that the colonies in cultures from guinea pigs were quite small and flat and tended to coalesce, and by the physical condition of the growth, which was moist and glistening in fresh cultures. A portion of the growth could be easily removed with a platinum loop and brought into a state of fine subdivision in bouillon.

Group II (consisting of human cultures F T, F L, S E No. 3, G D, 12124, C No. 1, D T, and No. 50) exhibited usually in original cultures, round distinctly elevated colonies, and in subcultures the growth was more abundant than in the cultures of Group I. It was dry, rather difficult to remove with a platinum wire, and not infrequently exhibited a sheen on the surface. The growths of this group on egg were brought into a fine state of subdivision with considerable difficulty, it being practically impossible to get rid of the many small flakes which clung together and could not be broken up. Smith noted, in regard to serum cultures of his bovine and human organisms, that the bovine cultures could be brought into a finer state of subdivision than the human cultures.

Serum cultures.—During the length of time they have grown on serum (about 5 months), the distinction between the two groups has not been so marked as could be noted in the case of egg cultures. In fact, the differences between the two sets of cultures were not sharply defined, there being really more differences between the individuals of each than were found between the groups as a whole. In the case of Group I in C No. 81, E T, and bovine III, the gross appearances agreed fairly well, although there were differences noted in the several transfers which were mainly attributable to variations in the amount of growth. Culture C No. 4, grew very poorly on the serum, almost always presenting simply a thin film of growth, giving a ground-glass appearance to the culture. In Group II several of the members grew in a manner practically indistinguishable from the bovine cultures, while culture D T gave from the beginning a heavy abundant white growth.

To sum up the gross differences observed in cultures upon hardened dog's serum, they seem to depend upon the luxuriance of the growth rather than upon any qualitative variation, with the exception of culture C No. 4, in which instance it was evidently of a different character. It is quite manifest that in the case of two growths, both in themselves white, the one which is the most abundant will be opaque and will therefore present a white color, whereas the other, growing less vigorously and forming a film which is translucent, will appear to be gray. The varying color of the cultures upon serum, therefore, seems to be due wholly to the amount of growth which takes place.

The serum was hardened at 74° to 76° C., and identical lots of serum were used for all comparative examinations. The cotton plugs were paraffined, as in the case of the egg cultures.

Microscopic appearance of the cultures.—The method of making the preparations for microscopic examination was as follows: A small portion of the culture to be examined was spread in a thin film on a cover-slip and allowed to dry in the air. The preparation was then fixed in a hot-air oven at a temperature of 112° to 117° C. for 40 minutes. It was stained with cold carbol-fuchsin for 30 minutes and, after rinsing in distilled water, the preparation was allowed to remain 30 seconds in 10 per cent sulphuric acid (by volume); then it was thoroughly rinsed with water, dried, and mounted in Canada balsam. This method was followed in all comparative examinations, and, in addition, in order to insure identically the same treatment, all of the preparations to be examined were fixed together in the same oven for the same length of time.

Microscopic examination of egg cultures.—The comparative examinations of cultures on egg have failed to bring out any marked differences in the morphology of the various strains of tubercle bacilli, although it is quite possible that certain differences which I have found to be very slight now might, after a prolonged period of cultivation on this medium, become more marked. Generally speaking, all of the individuals from all of the cultures were well stained, short, and rather thick. Very little tendency to beading was noticed in any of them, and they were all quite uniform in size, long and short forms not being seen together in any of the preparations. The usual length of the individual organisms was 1.5 to 2 μ. D T, human, and C No. 1, human, might be put down as exceptions, for they have usually been from 2 to 2.5μ in length, but with no visible tendency to lengthen during cultivation.

Microscopic examination of serum cultures.—The cultures C No. 81, bovine, E T, human, and C No. 4, human, have been grown also on dog's serum. It has already been shown that these three cultures were practically identical in the gross appearance of their growths on egg, and, as will appear later, they corresponded so closely in other properties that it was thought advisable to study carefully their characters when grown on hardened dog's serum. Their cultivation upon this medium has now extended over a period of about 5 months. During this time cover-glass preparations have been made several times exactly according to the method described for the egg cultures. Cultures C No. 81, bovine, and E T, human, are now, after 5 months of cultivation, morphologically indistinguishable. Both organisms are short and thick, 1.5 to 2 μ long, generally straight, and uniformly stained. In culture C No. 4 the individuals are perceptibly longer and slenderer and are usually stained evenly, though in some of the cultures beading has been quite prominent. The length of these organisms is from 2 to

2.5 μ. The differences pointed out have existed since the first generation on dog's serum and have not increased during cultivation through five successive generations. The accompanying photographs (Pls. I and II, p. 10) illustrate fairly well the morphological characters of cultures C No. 81, E T, C No. 4, and S E No. 3 when grown on hardened dog's serum.

The following summary of results will serve to bring together the salient features of the growth of the various cultures on egg and blood serum:

(1) The gross characters of the various cultures on egg were of such a nature that they could be separated into two groups. Group I comprised the most virulent cultures, as is shown later in this paper, and the differences between the two groups were sufficiently marked to make it possible to predict that certain cultures (on account of their resemblance to the bovine cultures on egg) would prove virulent for calves, which prediction was borne out by the results of subsequent inoculation experiments. Group I includes two bovine cultures and two human cultures. Group II includes the remaining human cultures.

(2) The microscopic characters of the various organisms cultivated on egg exhibited such slight differences that no sharp division line could be drawn. The bovine bacilli were indistinguishable from the majority of the human bacilli.

(3) The gross characters of the cultures on dog's serum did not permit of a division into two groups such as was possible with the egg cultures, the differences which were observed being in the quantity of growth rather than in its quality. There was one exception to this rule and that was in culture C No. 4, human, which always exhibited a thin film, resembling ground glass, never growing so luxuriantly as the others.

(4) The microscopic characters of the three cultures which were grown on dog's serum long enough to make a careful examination of them worth while; that is, cultures E T, C No. 4, human, and C No. 81, bovine, were not such as to permit of any sharp distinction between them, though culture C No. 4, was slightly longer from the first than the other two.

PATHOGENIC PROPERTIES.

GUINEA PIGS.—All of the animals which were used in these experiments were as vigorous and healthy as it was possible to obtain them. None had ever been used for any other purpose; and, after inoculation, they were kept in perfectly clean, separate cages, one guinea pig usually being placed in a cage with a rabbit which had received the same culture. The animals were not handled after inoculation, the main reliance being placed upon the length of life and the result of the macroscopic and microscopic examinations after death. The heaviest guinea pig weighed 664 grams and the lightest 424 grams, the average weight being 532 grams. All guinea pigs were inoculated subcutaneously on the inner side of the thigh. The doses given were as nearly uniform as it was possible to make them, the culture being prepared for inoculation in the same manner as that used by Smith, which is as follows: Particles of the culture were carefully transferred from the culture tube to the inside of a dry sterile test tube until sufficient was obtained to make the desired amount of suspension. By the use of a heavy platinum wire having a flattened spatula-like end, the clumps of bacilli were rubbed against the sides of the tubes until all were broken up and the bacilli smeared in a thin film on the glass. A small amount of sterile peptonized bouillon was now added and by means of the platinum wire the culture was mixed thoroughly with the fluid. More bouillon was added until the required dilution was obtained. For reasons previously stated, I have adopted for all inoculations a suspension which was made as nearly as possible equal in density to a 24-hours typhoid culture. A uniform dose of 0.5 c.c. was used.

The guinea pig which survived the longest was one which received human culture No. 50, the animal dying of generalized tuberculosis 61 days after inoculation. This culture, previous to its passage through the monkey, had been grown on artificial media for more than 8 years. One guinea pig inoculated with human culture C No. 1 lived 54 days, although another guinea pig of greater weight than the first one succumbed to an equal dose of the same organism in 31 days. The other animals died after inoculation at varying lengths of time ranging from 40 days downwards.

In Table I a summary of the inoculations and their results is given. For the sake of obtaining a better comparison in the discussion of these results, the various cultures have been divided into three groups. It must be understood, however, that this grouping is made in order that the results may be more clearly explained and not primarily on account of their different effect upon guinea pigs.

A few of the guinea pigs died within less than 2 weeks after inoculation, and unless these animals showed sufficient tuberculosis to account for their death they were not taken into consideration in computing the average length of life. Such animals are marked with a star in the tables.

Group I, bovine cultures C No. 81 and III.—The guinea pigs inoculated with these cultures had an average weight of 542 grams and lived after inoculation an average of 23⅔ days.

Group II, human cultures E T and C No. 4.—The guinea pigs inoculated with these cultures had an average weight of 565 grams and lived an average of 22¼ days.

Group III, human cultures, No. 50, F L, S E No. 3, G D, 12124, C No. 1, and D T.—The guinea pigs inoculated with this group of cultures had an average weight of 530 grams and lived an average of 35½ days.

It will be seen that between Group I (bovine cultures C No. 81 and III) and Group II (human cultures E T and C No. 4) the differences in the length of life after inoculation are so slight that no separation based on this point would be possible. If anything, the human cultures C No. 4 and E T might be considered as slightly more virulent; for, although the guinea pigs of this group averaged 23 grams heavier, they lived an average of 1 day less.

The cultures of Group III are apparently distinctly less virulent for guinea pigs than those of Groups I and II, the animals having survived an average of 12 days longer than the bovine guinea pigs, which were of greater average weight.

The similarities and differences between these three groups appear also in the results of the postmortem macroscopic and microscopic examinations. The spleen, liver, and lungs are the organs which are principally affected in guinea pigs, and when the inoculation has been made subcutaneously on the inside of the thigh the lungs are the last of those organs to exhibit signs of the disease. For this reason the time required for the lungs to become involved may be taken as an index of the rate of progress of the disease, and I have considered the tuberculosis as generalized only when the lungs as well as the liver and spleen are affected.

In the case of the animals inoculated with cultures of Group III, the disease was generalized in all of those which survived 31 days or more. In those which survived longest the characteristic yellowish areas in the liver and the tubercles in the lungs were largest and most numerous, and the spleen was also most extensively diseased. In the case of those animals inoculated with the cultures of the Groups I and II the disease was generalized in all those which survived more than 23 days. None of the animals inoculated with these cultures lived more than 28 days, and only in those which survived the inoculation the longest were the large, yellowish, bile-stained areas in the liver very prominent. That organ, while showing in those which died first some such changes, had usually the appearance of having undergone very rapid degenerative changes, which, while just as extensive, had not developed so far as in those animals of Group III which lived for a longer time.

The impression conveyed, therefore, by the macroscopic appearance at the autopsies on guinea pigs was that the disease induced by the cultures of Groups I and II was more rapid in its progress than that induced by the cultures of Group III, and that death resulted from the extensive interference with the functions of the various organs before the tuberculous process had really reached its height. In the case of Group III the disease seems to have been disseminated less rapidly, the organs being able to perform their functions less perfectly, of

DESCRIPTION OF PLATE III.

FIG. 1.—Section of lung of guinea pig No. 2077. Animal was inoculated with bovine tubercle culture C No. 81. Tissue hardened in alcohol and embedded in celloidin. Stained with hematoxylin and carbol-fuchsin as described in text. Drawn with camera lucida. Magnified 1,450 diamaters.

FIG. 2.—Liver of guinea pig No. 2077. Same as fig. 1.

FIG. 3.—Section of lung of guinea pig No. 2592. Animal was inoculated with human tubercle culture C No. 4. Tissue hardened in alcohol and embedded in celloidin. Stained with hematoxylin and carbol-fuchsin. Drawn with camera lucida. Magnified 1,450 diameters.

FIG. 4.—Liver of guinea pig No. 2592. Same as fig. 3. Drawings by W. S. D. Haines.

DESCRIPTION OF PLATE IV.

FIG. 1.—Section of lung of rabbit No. 538. Animal was inoculated with bovine tubercle culture C No. 81. Tissue hardened in alcohol and embedded in celloidin. Stained with hematoxylin and carbol-fuchsin. Drawn with camera lucida. Magnified 1,450 diameters.

FIG. 2.—Liver of rabbit No. 538. Same as fig. 1. Drawings by W. S. D. Haines.

DESCRIPTION OF PLATE V.

FIG. 1.—Section of lung of rabbit No. 488. Animal was inoculated with human tubercle culture C No. 4. Tissue hardened in alcohol and embedded in celloidin. Stained with hematoxylin and carbol-fuchsin. Drawn with camera lucida. Magnified 1,450 diameters.

FIG. 2.—Liver of rabbit No. 488. Same as fig. 1. Drawings by W. S. D. Haines.

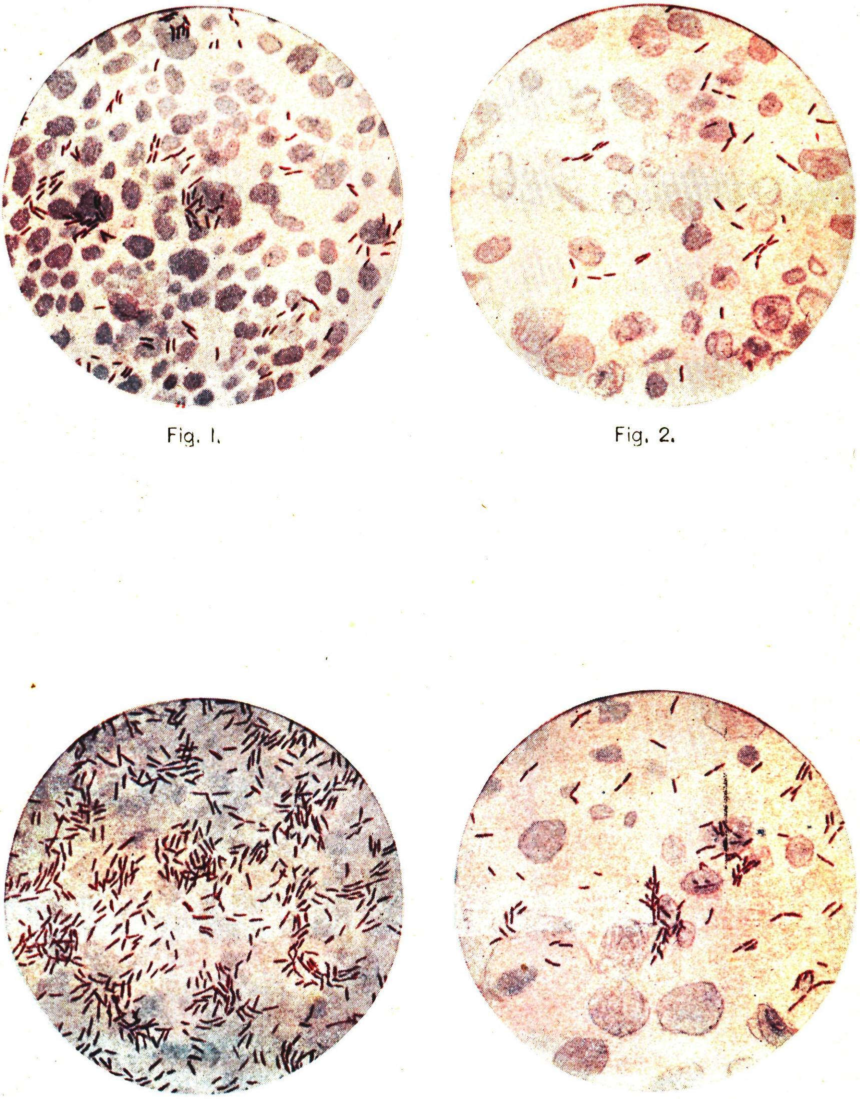

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Sections showing bacilli in tubercles from inoculated guinea pigs.
Figs. 1 and 2, bovine, Figs. 3 and 4, human tuberculosis.

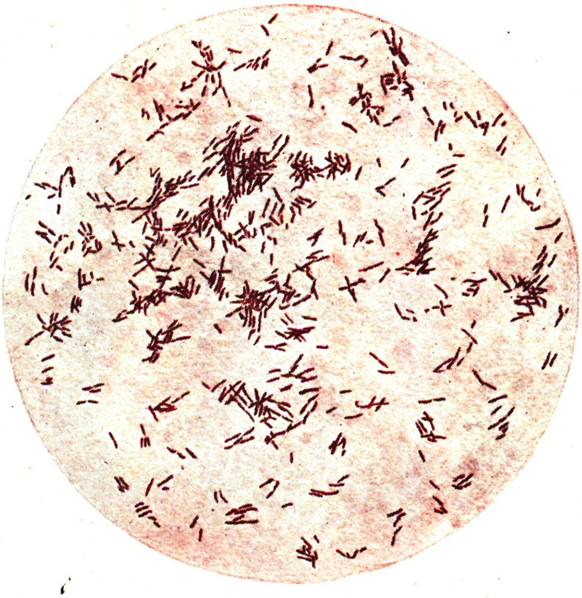

Fig. 1.

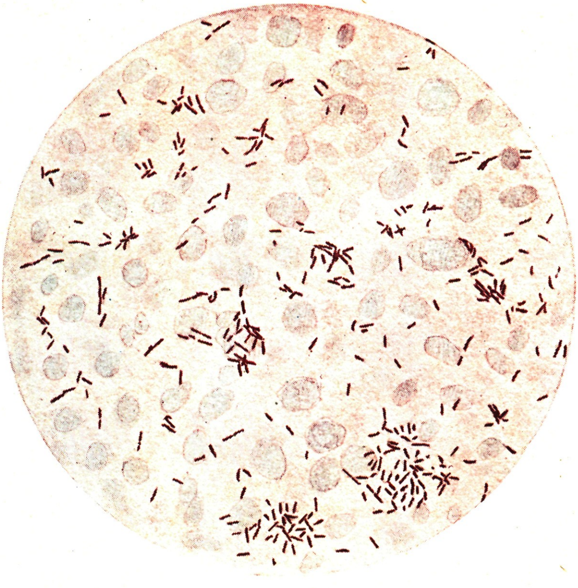

Fig 2.

Sections showing bacilli in tubercles from rabbits inoculated with bovine tuberculosis.

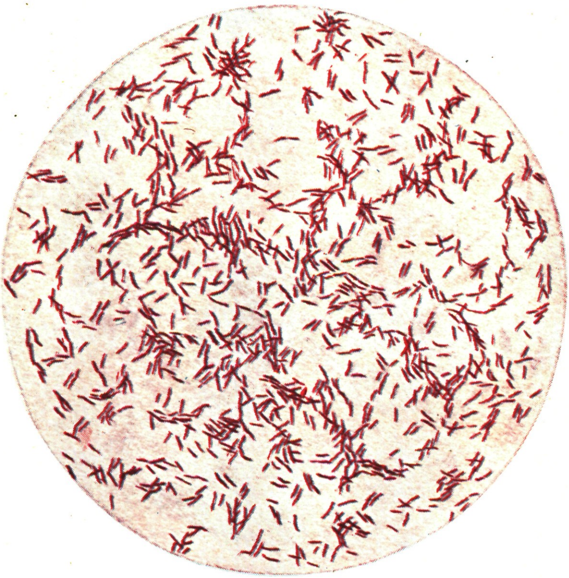

Fig. 1.

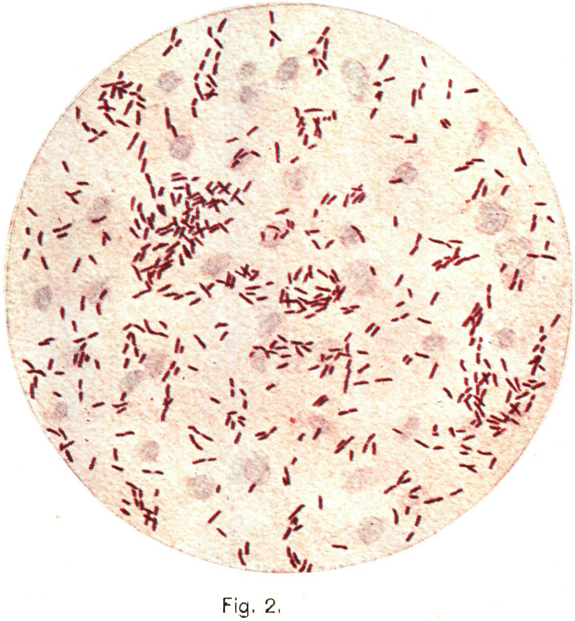

Fig. 2.

Sections showing bacilli in tubercles from rabbits inoculated with human tuberculosis.

course, but sufficiently long for the tubercles to reach a further stage of development.

The microscopic examination of the tissues from the various animals was made as complete as possible, sections being made of the spleen and liver, and of the lungs also whenever any disease of those organs was present. The methods used for preparing the tissues and staining were as follows: The hardening was done with 80 per cent alcohol and the tissues embedded in celloidin. For the examination of tissue changes the sections were stained with Delafield's hematoxylin and with eosin, and mounted in Canada balsam after clearing with creosote. For the demonstration of tubercle bacilli the sections were usually stained lightly with hematoxylon and 30 minutes in cold carbol-fuchsin. The removal of the excess of carbol-fuchsin was effected by means of Orth's discharging fluid,[a] the section being subsequently immersed in water containing a trace of ammonia to restore the blue to the cell nuclei. In some instances the hematoxylin was not used, the counterstaining being accomplished by means of methylene blue after the use of the carbol-fuchsin. All the sections were cleared in creosote or clove oil and mounted in Canada balsam.

In the microscopic examination of the tissues, special attention was paid to the following points: 1, the number and size of the tubercles; 2, the condition of the tubercles—whether they showed necrosis of the center and whether there were any evidences of retrogressive changes indicating decided resistance on the part of the body to the invasion of the tubercle bacillus; 3, the number of tubercle bacilli in the lesions.

First of all it may be stated that, in the microscopic as in the macroscopic examinations, no essential difference in the nature of the pathologic changes could be noted in the different guinea pigs. The differences observed were only such as are always found in tubercles which have reached varying stages in their development. The histologic changes in the organs of animals inoculated with cultures of Groups I and II were practically identical in those animals which survived approximately the same length of time, while in those animals inoculated with Group III which died at the end of the same number of days the lesions were always less advanced, about 8 days longer being required for cultures of this group to produce lesions approximating those produced by Groups I and II.

In none of the guinea pigs were the lung tubercles sufficiently far advanced to show a characteristic necrosis of the central portion, although in a few instances its beginning was indicated by a fragmentation of the cell nuclei. The liver and spleen in all the inoculated guinea pigs contained tubercles with characteristic central necrosis and usually in the liver the hepatic cells outside of the zone of the tubercles showed greater or less degenerative changes, indicated by obliteration of the nuclei and a fatty degeneration. In the spleen the tubercles were not well defined, there being found, as a rule, large irregular areas of coagulation necrosis. Tubercle bacilli were most abundant in these necrotic areas.

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[a] Hydrochloric acid, 1 c. c.; 70 per cent alcohol, 99 c. c.

Tubercle bacilli were much more abundant in the tubercles in those guinea pigs inoculated with the cultures of Groups I and II than in those inoculated with any of the members of Group III. There were no constant differences between the bovine cultures C No. 81 and III, on the one hand, and the human cultures C No. 4 and E T, on the other. In the sections tubercles would occasionally be found after inoculation with the bovine cultures which contained more tubercle bacilli than certain tubercles in guinea pigs inoculated with C No. 4 and E T, and the exact reverse was occasionally noted. Such differences, however, were not marked, and no distinction, based on the number of bacilli in the tubercles, could be made between bovine cultures C No. 81 and III, on the one hand, and human cultures C No. 4 and E T, on the other. The accompanying drawings (Pl. III) illustrate very well the number of tubercle bacilli in the central portion of tubercles after inoculation of guinea pigs with bovine culture C No. 81 and the most virulent human cultures.

As a result of the guinea-pig inoculations just described, the following conclusions may be formulated, although they are based upon such slight differences that the distinctive features might be entirely obliterated by a series of experiments in which the animals used were appreciably lighter or where the dose of culture was appreciably greater:

(1) There was a sufficient variation in the virulence of the human cultures for guinea pigs to permit of their provisional separation into two groups.

(2) In those animals inoculated with the most virulent group of human cultures the disease was marked by its rapid progress (death in less than 30 days) and by the relatively greater number of tubercle bacilli in the lesions.

(3) There were no differences observed in the effect produced by the two most virulent human cultures and the two bovine cultures on guinea pigs. In both the disease was rapid in progress and in both tubercle bacilli were very numerous in the lesions.

(4) As a general proposition it may be stated that in the case of guinea pigs weighing over 400 grams and inoculated subcutaneously with 0.5 c. c. of a suspension having a density of a 24-hours typhoid culture a difference of from 100 to 150 grams in weight did not appear to influence the length of life of the animals.

Rabbits.—Aside from their effect upon cattle, it is upon their virulence for rabbits that Smith has laid particular stress in defining the differences between human and bovine tubercle bacilli, and in the inoculation experiments herein recorded every endeavor has been made to follow his methods closely, the only differences being in the culture medium used (egg instead of blood serum) and in the fact that, instead of favoring the human cultures by using lighter animals for them, I have endeavored to use rabbits for the human cultures which were equally as heavy or heavier than those which were inoculated with the bovine cultures. A glance at Table II will show that in all cases where duplicate inoculations were made the animals inoculated with the human cultures had a greater average weight than those inoculated with the bovine cultures.

The cultures were prepared for inoculation in the same manner as for the guinea-pig experiments. A uniform dose of 0.5 c. c. was used and all inoculations were made into an ear vein. No rabbits weighing less than 1,350 grams were used, and after inoculation they were not disturbed—no attempt being made to take temperatures or to weigh the animals.

An examination of the table will show that the various cultures may be readily separated into two groups, the separation being based upon the length of life and the gross lesions observed at the autopsy. The cultures of Group I, consisting of bovine cultures C No. 81 and III and human cultures C No. 4 and E T, caused the death of all rabbits within 20 days, the autopsy revealing always a severe generalized tuberculosis. The rabbits inoculated with the cultures of Group II, on the contrary, lived an average of 105¾ days (one animal inoculated with culture C No. 1 is still living at the end of a year). Five rabbits of this group died within 48 days after inoculation, 2 of this number succumbing on the twenty-seventh day. The rabbits of this group which succumbed before the forty-eighth day presented a very much less severe tuberculosis, however, than those inoculated with Group I. The gross lesions observed in the rabbits inoculated with the cultures of Group I presented at death a typical picture of a severe rapidly progressive miliary tuberculosis. The lungs were literally filled with minute tubercles 1 mm. or less in diameter, most of which showed a distinctly white necrotic center. The lungs did not collapse when the chest cavity was opened, the disease being so extensive that the lobes were almost completely solidified. The liver was rather large, and minute grayish points could usually be seen scattered over its surface. The spleens of all the animals were much enlarged, being usually from two to four times the normal size, and having scattered over their surfaces minute grayish areas, which were frequently so numerous as to give a steel-gray color to the organ, which, owing to the congestion, would otherwise have been dark red. The kidneys frequently showed on their surface numerous small grayish points quite similar to those seen on the liver and spleen.

The severe involvement of the lungs and spleen was always visible after inoculation with any member of Group I. The liver and kidney lesions were not always to be seen, but they were present in at least one of the animals inoculated with each of these cultures. With respect to their virulence for rabbits and the gross lesions produced, there was no observable difference between the human cultures C No. 4 and E T and the bovine cultures C No. 81 and III.

The cultures of Group II (consisting of cultures F T, F L, S E No. 3, G D, 12124, C No. 1, D T, and 50) showed sharp differences from Group I in the lesions which they produced, although there were considerable variations in the individual rabbits inoculated with the members of Group II. As has been remarked before, the rabbits inoculated with Group II had an average life of 105¾ days against an average of less than 20 days for those inoculated with Group I. Certain of the rabbits of Group II, however, survived only 27 days, and, in all, 5 died within 48 days. As the pathologic changes noted in those rabbits of this group which died before the forty-eighth day differed markedly from those seen in the rabbits which had survived a longer time, it will be better to consider the gross changes induced by Group II in two subgroups. Those rabbits of the first subgroup which succumbed within 48 days presented at the autopsy lesions which were very slight when compared with those produced by Group I and which did not seem sufficient to cause death. Indeed, the early death in 2 of the 5 cases might be attributed to a coccidium infection which was present in the livers of both. As to the lesions of tuberculosis seen, all of the 5 rabbits agreed fairly well. The lungs showed slight changes. Sprinkled over their surface were a number of small grayish, almost invisible points usually not more than 0.5 millimeter in diameter. These minute foci showed no coagulation necrosis and were apparently not numerous enough to interfere seriously with the normal functions of the lung, for the tissue outside of the minute tubercles appeared to be perfectly healthy, the lungs collapsing normally when the chest cavity was opened. These areas in the lungs correspond closely with those described by Smith for his sputum rabbits. They become white and plainly visible when immersed in alcohol. In a few of these rabbits some minute grayish points could be seen on the surface of the liver. The spleen and kidneys were normal in size and appearance. Those rabbits inoculated with Group II and which lived a longer time (forming the second subgroup) exhibited lesions which differed markedly from those noted in the rabbits which died at an earlier date, and they were also separated sharply in their macroscopic characters from those produced by Group I. In the rabbits of this latter subgroup, all of which lived more than 79 days, there was extensive involvement of the lungs and kidneys. In the lungs there were large white irregular areas of coagulation necrosis, some having a diameter of 1 cm. The necrotic areas were so extensive as to necessarily interfere seriously with the function of the lungs. In some of the rabbits which lived the longest a softening had taken place in the necrotic areas and abscesses were produced which contained a rather soft white pus. The liver and spleen in all these animals were apparently normal, while the kidneys were, without exception, extensively diseased, showing on the surface and in the body of the organ white necrotic areas of varying size, some of which had softened.

A careful histologic examination was made of the lungs of all the inoculated rabbits, and, in the case of most of them, the liver, spleen, and kidneys were also studied. The method of embedding and staining was the same as that pursued in the examination of tissues from the guinea pigs. As a result of this microscopic work it is again possible to separate the various cultures into two groups. The lesions caused by Group I are characterized by the distinct necrosis of the central portion of even very young tubercles and by the very great number of tubercle bacilli in them. In the lungs the bacilli were present in enormous numbers. (Pls. IV, V.) This group (I) comprises bovine cultures No. 81 and III and human cultures E T and C No. 4.

The cultures of Group II are the remaining human cultures. In those animals which died within 7 weeks necrotic changes were absent, the tubercles being marked by an accumulation of epithelioid cells; occasionally giant cells could be seen. Tubercle bacilli were usually scarce, only a few being visible in each nodule. The tubercles were fairly numerous in the lungs, but very scarce in the liver, spleen, and kidney. In those rabbits which lived more than 7 weeks the necrotic changes were marked, involving large areas, but the tubercle bacilli were not numerous, except in those areas which had broken down. There they were usually present in considerable numbers.

To recapitulate, the intravenous inoculation of rabbits served to separate the cultures into two groups. Group I (human cultures C No. 4, and E T, and bovine cultures C No. 81 and III) caused the death of the rabbits within 20 days, due to a severe generalized tuberculosis, a necrosis of the central portion of the tubercles being marked, and large numbers of tubercle bacilli being present in the lesions. Group II (human cultures No. 50, F L, F T, S E No. 3, G D, 12124, C No. 1, and D T) caused the death of rabbits after an average of 105 days. Those rabbits which died in less than 48 days did not show sufficient tuberculosis to cause death; necrosis was practically absent and very few tubercle bacilli were found. Those that lived longer than 48 days showed extensive necrotic changes, but in most of the tubercles very few bacilli could be seen.

From the results of the rabbit inoculations which have just been described, the following conclusions may be drawn. It must be remembered, however, that they are based upon results obtained when the rabbits weighed over 1,300 grams, and when the dose was the same as that already noted. Any considerable variation in either of these two factors might modify the results considerably.

(1) Mammalian tubercle bacilli may be divided into two classes: (*a*) Those which cause the death of rabbits in 20 days, due to a severe generalized tuberculosis; and (*b*) those which kill only after several months, or when an earlier death takes place the lesions produced are not severe and necrotic changes are conspicuous by their absence.

(2) The lesions resulting from the inoculation with the virulent cultures are characterized by their wide distribution, rapid necrosis, and by the very great numbers of bacilli in the lesions.

(3) The lesions resulting from the inoculation with the less virulent cultures are characterized by the absence of necrosis in the tubercles in those rabbits which died within 7 weeks, and by the large conglomerate tubercles with extensive necrosis seen in those rabbits which survived for a longer time. Tubercle bacilli were found usually in very small numbers.

(4) Tubercle bacilli of bovine origin, in their action upon rabbits, were indistinguishable from the most virulent human cultures (E. T. and C No. 4).

GENERAL CONCLUSIONS.

The foregoing experiments have been especially striking on account of the widely varying virulence among the human tubercle cultures, and by reason of the fact that those human cultures showing the highest degree of virulence were indistinguishable from the bovine cultures with which they were compared. It is also interesting to note that those human cultures which were identical with the bovine cultures in their virulence for rabbits were also capable of causing a generalized tuberculosis in calves. And in addition only those cultures which caused the death of rabbits within 20 days were capable of bringing about a generalized tuberculosis in calves after subcutaneous inoculation. There seems to be no reason whatever for making a distinction between the two bovine cultures and human cultures C No. 4 and E T, so far as their action upon guinea pigs and rabbits is concerned, and as a result of our experiments the following general conclusions seem unavoidable:

(1) Certain tubercle bacilli of human origin are indistinguishable either culturally, morphologically, or with regard to their virulence for rabbits and guinea pigs from certain tubercle bacilli of bovine origin.

(2) There is considerable variation in the virulence of human tubercle bacilli for rabbits and guinea pigs.

Until we know what influence a residence in the human body exerts upon bovine tubercle bacilli we can not determine accurately the proportion of cases of human tuberculosis which result from infection from cattle.

I am inclined to the belief, from the experiments here presented and from those of Ravenel, Vagedes, Fibiger & Jensen, and others, that in bovine and human tuberculosis we have to do with organisms differing usually in virulence, but between which there is no other essential distinction.

More detailed study of tubercle bacilli derived from accidental inoculation of men with bovine virus is needed, together with a more extended examination of bovine bacilli derived directly from cattle.

TABLE I.—*Results of inoculating guinea pigs with cultures of human and bovine origin.*

Culture.	Age of culture used.	Total age of culture used.	Number of generation on artificial media.	Culture medium.	Dose of culture.	Mode of inoculation.	No. of animal.	Initial weight of animal.	Date of inoculation, 1902.	Result of inoculation.	Remarks.
	Days.	*Days.*			*c. c.*			*Gms.*			
F T, human	30	138	Second	Egg	0.5	Subcutaneous	G. P. 2072.	472	Aug. 21	Died Aug. 30, 1902, without lesions of tuberculosis.	[a] This animal probably died of pneumonia.
F L, human	30	136	Third	...do	.5	.....do	G. P. 2073.	527	....do	Died Sept. 23, 1902; generalized tuberculosis.	Lived 32 days.
Do	28	216	Fifth	...do	.5	.....do	G. P. 2632.	664	Nov. 11	Died Dec. 4, 1902; tuberculosis of liver and spleen.	Lived 23 days.
S E No. 3, human	30	56	Second	...do	.5	.....do	G. P. 2075.	527	Aug. 21	Died Sept. 28, 1902; generalized tuberculosis.	Lived 37 days.
G D, human	28	28	First	...do	.5	.....do	G. P. 2078.	602	....do	Died Oct. 1, 1902; generalized tuberculosis.	Lived 40 days.
12124, human	58	58	....do	...do	.5	.....do	G. P. 2554.	522	Nov. 11	Died Dec. 2, 1902; tuberculosis of liver and spleen.	Lived 21 days.
C No. 1, human	22	130	Second	...do	.5	.....do	G. P. 2079.	615	Aug. 21	Died Sept. 22, 1902; generalized tuberculosis.	Lived 31 days.
Do	57	57	First	Dog's serum.	.5	.....do	G. P. 1329.	424	Mar. 25	Died May 19, 1902; generalized tuberculosis.	Lived 54 days.
Do	57	57	....do	...do	.5	.....do	G. P. 1332.	460	....do	Died Apr. 1, 1902; no tuberculosis of viscera.	[a] Lived 6 days.
D T, human	30	64	Second	Egg	.5	.....do	G. P. 2074.	477	Aug. 21	Died Sept. 22, 1902; generalized tuberculosis.	Lived 31 days.
Do	28	145	Fifth	...do	.5	.....do	G. P. 2633.	500	Nov. 11	Died Dec. 6, 1902; tuberculosis of liver and spleen.	Lived 25 days.
50, human	48	48	First	Dog's serum.	.5	.....do	G. P. 1330.	480	Mar. 25	Died Apr. 5, 1902; beginning tuberculosis of liver and spleen.	[a] Lived 11 days.
Do	48	48	....do	...do	.5	.....do	G. P. 1331.	448	....do	Died May 26, 1902; generalized tuberculosis.	Lived 61 days.

E T, human	22	82	Second	Egg	.5	....do	G. P. 2076.	501	Aug. 21	Died Sept. 15, 1902; generalized tuberculosis.	Lived 25 days.
Do	50	156	Fourth	...do	.5	....do	G. P. 2591.	621	Nov. 4	Died Nov. 27, 1902; generalized tuberculosis.	Lived 23 days.
C No. 4, human	18	63	Third	...do	.5	....do	G. P. 2266.	493	Oct. 3	Died Oct. 19, 1902; tuberculosis of liver and spleen.	Lived 16 days.
Do	31	95	Fourth	...do	.5	....do	G. P. 2592.	648	Nov. 4	Died Nov. 29, 1902; generalized tuberculosis.	Lived 25 days.
C No. 81, bovine	30	70	Second	...do	.5	....do	G. P. 2077.	561	Aug. 21	Died Sept. 19, 1902; generalized tuberculosis.	Lived 28 days.
Do	70	143	Third	...do	.5	....do	G. P. 2593.	584	Nov. 4	Died Nov. 25, 1902; generalized tuberculosis.	Lived 21 days.
III, bovine	57	57	First	Dog's serum.	.5	....do	G. P. 1333.	480	Mar. 25	Died Apr. 16, 1902; tuberculosis of liver and spleen.	Lived 22 days.
Do	57	57	....do	....do	.5	....do	G. P. 1334.	428	....do	Died Apr. 5, 1902; beginning tuberculosis of liver and spleen.	[a] Lived 11 days.

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[a] Not considered in computing average length of life, not having shown sufficient tuberculosis to account for death.

TABLE II.—*Results of inoculating rabbits with cultures of human and bovine origin.*

Culture.	Age of culture used.	Total age of culture used.	Number of generation on artificial media.	Culture medium.	Dose of culture.	Mode of inoculation.	No. of animal.	Initial weight of animal.	Date of inoculation, 1902.	Result of inoculation.	Results.
	Days.	*Days.*			*c. c.*			*Gms.*			
F T, human	30	138	Second	Egg	0.5	Intravenous	Rab. 462	2,050	Aug. 21	Died Dec. 31, 1902; tuberculosis of lungs and kidneys.	Lived 130 days.
F L, human	30	136	Third	do	.5	do	Rab. 465	1,520	do	Died Nov. 9, 1902; generalized tuberculosis.	Lived 79 days; weight at death, 1,191 grams.
Do	28	216	Fifth	do	.5	do	Rab. 512	1,640	Nov. 11	Died Dec. 13, 1902; tuberculous lesions in lungs.	Lived 32 days; tuberculosis very slight.
S E No. 3, human	30	56	Second	do	.5	do	Rab. 463	1,975	Aug. 21	Died Oct. 9, 1902; generalized tuberculosis.	Lived 48 days; very mild type of disease.
G D, human	28	28	First	do	.5	do	Rab. 468	2,300	do	Died Feb. 10, 1903; tuberculosis of lungs and kidneys.	Lived 170 days.
12124, human	58	58	do	do	.5	do	Rab. 511	1,350	Nov. 11	Died Dec. 27, 1902; tuberculosis of lungs.	Lived 46 days; coccidium infection of liver.
C No. 1, human	22	130	Second	do	.5	do	Rab. 467	2,030	Aug. 21	Chloroformed Mar. 6, 1903; tuberculosis of lungs and kidneys.	Lived 193 days; weight at death, 1,675 grams.
Do	57	57	First	Dog's serum.	.5	do	Rab. 287	1,925	Mar. 25	Still living Mar. 10, 1903	Apparently in perfect health.
Do	57	57	do	do	.5	do	Rab. 289	2,175	do	Died Oct. 1, 1902; tuberculosis of lungs and kidneys.	Lived 186 days.
D T, human	30	64	Second	Egg	.5	do	Rab. 464	2,070	Aug. 21	Died Mar. 2, 1903; tuberculosis of lungs and kidneys.	Lived 189 days.
Do	28	145	Fifth	do	.5	do	Rab. 513	1,350	Nov. 11	Died Dec. 8, 1902; slight tuberculosis of lungs.	Lived 27 days.
50, human	48	48	First	Dog's serum.	.5	do	Rab. 141	1,627	Mar. 25	Died Apr. 22, 1902; generalized tuberculosis.	Lived 27 days; a very mild type of disease.
Do	48	48	do	do	.5	do	Rab. 295	2,450	do	Died Aug. 12, 1902; tuberculosis of lungs and kidneys.	Lived 142 days.

E T, human	22	82	Second	Egg	.5	.....do	Rab. 466	1,380	Aug. 21	Died Sept. 6, 1902; generalized tuberculosis.	Lived 16 days; severe type of disease.
Do	50	156	Fourth	...do	.5	.....do	Rab. 489	1,910	Nov. 4	Died Nov. 19, 1902; generalized tuberculosis.	Lived 15 days; severe type of disease.
Ç No. 4, human	18	63	Third	...do	.5	.....do	Rab. 505	1,594	Oct. 3	Died Oct. 17, 1902; generalized tuberculosis.	Lived 14 days; severe type of disease.
Do	31	95	Fourth	...do	.5	.....do	Rab. 488	1,922	Nov. 4	Died Nov. 22, 1902; generalized tuberculosis.	Lived 18 days; severe type of disease.
C No. 81, bovine	30	70	Second	...do	.5	.....do	Rab. 460	1,370	Aug. 21	Died Sept. 4, 1902; generalized tuberculosis.	Lived 14 days; severe type of disease.
Do	70	143	Third	...do	.5	.....do	Rab. 538	1,612	Nov. 4	Died Nov. 21, 1902; generalized tuberculosis.	Lived 17 days; severe type of disease.
III, bovine	57	57	First	Dog's serum.	.5	.....do	Rab. 139	2 038	Mar. 25	Died Apr. 14, 1902; generalized tuberculosis.	Lived 20 days; severe type of disease.

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U. S. DEPARTMENT OF AGRICULTURE,
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EXPERIMENTS CONCERNING TUBERCULOSIS,

UNDER THE SUPERVISION OF

THE BIOCHEMIC DIVISION.

PART II.

The Comparative Virulence of Human and Bovine Tubercle Bacilli for Some Large Animals.

BY

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U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY—Bulletin No. 52, Part II.
D. E. SALMON, D. V. M., Chief of Bureau.

EXPERIMENTS CONCERNING TUBERCULOSIS,

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., November 28, 1904.

Sir: I have the honor to transmit herewith a manuscript entitled "The comparative virulence of human and bovine tubercle bacilli for some large animals," by Drs. E. A. de Schweinitz, M. Dorset, and E. C. Schroeder.

When the experimental work described in this manuscript had been practically completed, Doctor de Schweinitz, who was at that time chief of the Biochemic Division of this Bureau, was taken suddenly ill and, to our great sorrow, died. This unfortunate occurrence threw upon the remaining authors of this paper the entire burden of its preparation, together with the formulation of the conclusions expressed therein. In view of this fact Doctor de Schweinitz should be given credit for the active part taken by him in the prosecution of these experiments, but should not be held responsible for the conclusions drawn therefrom.

This manuscript describes the inoculation of cattle, hogs, and monkeys with tuberculous virus of both human and bovine origin and bears directly upon the very important question of the infectiousness of bovine tuberculosis for human beings. I recommend that this manuscript, together with the accompanying illustrations, which are necessary to a thorough understanding of the text, be published as Part II of Bulletin No. 52 of this Bureau.

Respectfully,

D. E. Salmon,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture

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Part II.—THE COMPARATIVE VIRULENCE OF HUMAN AND BOVINE TUBERCLE BACILLI FOR SOME LARGE ANIMALS.

By E. A. de Schweinitz, Ph. D., M. D., Marion Dorset, M. D., and E. C. Schroeder, M. D. V.

The experiments described in this part of Bulletin No. 52 deal almost exclusively with (1) the infectiousness of tubercle bacilli of human origin for hogs and cattle and (2) the comparative virulence of human and bovine tuberculous virus for monkeys. The preliminary notes, indicating the important features of these experiments, have already been published, and it is our desire to present here the details of the experiments, together with a brief review of some of the literature which bears more or less directly upon the work we have done.

PLAN OF THE EXPERIMENTS.

The original plan adopted for these experiments contemplated the following lines of work:

(1) The isolation of fresh cultures of human tuberculosis from sputum and from cases of generalized tuberculosis in adults and children, obtaining whenever possible material from cases which indicated an involvement of the abdominal viscera.

(2) The inoculation of cattle and hogs intravenously and subcutaneously with the cultures or material so obtained.

(3) The feeding of human tuberculous material to calves.

(4) The isolation of fresh cultures of bovine tubercle bacilli and their use for comparison with the cultures from human beings mentioned above.

(5) The subcutaneous and intravenous inoculation of cattle and hogs with such bovine cultures or with bovine tuberculous material.

(6) The inoculation of cattle with bovine and human tubercle bacilli which had been under cultivation in the laboratory for some years and which possessed probably a comparatively low degree of virulence.

It was our plan to trace the histories of the subjects from whom the human tuberculous material was taken and to give complete autopsy records of these persons. The animals which succumbed to inoculation were to be carefully examined after death, and the lesions found were to be studied microscopically in order to determine if possible whether or not the disease produced was of a progressive character.

Before the experiments progressed very far it became evident that certain of the projected details which seemed quite desirable could not be fully carried out. The subjects from whom the tuberculous material was obtained belonged mostly to the pauper classes, and it was impossible to ascertain with any degree of certainty the sources of their food supply. In addition, the family histories were valueless almost without exception. In other cases from which sputum was obtained no autopsy was made when the subject finally died, owing usually to prejudice on the part of relatives. The records of most of the cases of human tuberculosis are therefore very unsatisfactory in so far as they concern the mode of infection.

But let it be remembered that the primary object of these experiments is to determine whether tuberculosis in cattle may be brought about by tubercle bacilli which have caused serious or fatal disease in man. The source from which the human beings were infected may be of great importance when it can be traced, but it is not necessary to a solution of the problem which we have undertaken.

METHOD OF OBTAINING THE CULTURES.

Before discussing the general features of the experiments it should be stated that all the cultures used were isolated from guinea pigs which had been inoculated with material from the various cases of human and bovine tuberculosis. As a rule, the cultures were obtained from the spleens of guinea pigs and grown on the egg medium, which is described in detail on page 9 of Part I of this bulletin. In the comparative inoculations the cultures were approximately of the same age and the doses given as nearly uniform as possible.

SOURCES OF HUMAN TUBERCULOUS MATERIAL.

As previously stated, we were unable to secure full data relative to the previous histories and autopsy conditions of many of the cases of human tuberculosis which supplied material for our investigations. In some instances the hospital records, although quite voluminous, were deficient in details of especial interest in experiments of this character; hence only such portions of them as have a direct bearing upon the origin or nature of the tuberculosis with which the several individuals were afflicted will be inserted in this bulletin.

We must express our sincere thanks to the physicians who kindly furnished us with material and records from the several hospitals in this city. The tissue obtained from the Government Hospital for the Insane was sent to us, together with the postmortem records, by

Dr. I. W. Blackburn, the pathologist of that institution. For the material from the Children's Hospital we are indebted to Doctors Grasty and Mason of the house staff, and for the sputum obtained from patients in the Washington Asylum Hospital to Doctor Price, house physician in the asylum.

(1) *Culture F T, human.*—This culture was obtained on egg from the lung of a guinea pig which had been inoculated intraabdominally with sputum from a 45-year-old negro woman whose occupation was that of a domestic. She was admitted to the Washington Asylum Hospital February 16, 1902, after a diagnosis of pulmonary tuberculosis with chronic interstitial nephritis had been made. There was nothing unusual during the course of her illness. She died February 23, 1902. No autopsy could be obtained.

(2) *Culture F L, human.*—This culture was obtained from the spleen of a guinea pig which had been inoculated intraperitoneally with sputum from a painter 40 years old. He was admitted to the Washington Asylum Hospital on June 26, 1902, after a diagnosis of pulmonary tuberculosis had been made. This patient died on November 4, 1902. Consent to a postmortem examination could not be obtained.

(3) *Culture S E No. 3, human.*—This culture was obtained from the spleen of a guinea pig which had been inoculated subcutaneously with a small fragment from the lung of a man 50 years old. This man was admitted to the Government Hospital for the Insane on July 28, 1890, suffering from chronic dementia. Owing to the patient's condition, no evidence could be obtained relative to his previous history. The illness which terminated in his death appeared to begin on September 3, 1901, when he had a chill followed by fever. From that date the temperature varied from 98° to 102° F. On May 15, 1902, the abdomen was distended and the patient vomited copiously. Death took place on May 16.

The autopsy revealed pleuritic adhesions of both lungs. In the left lung old tuberculous deposits were found; in the apex there were some small cavities; secondary tuberculous granulations were scattered throughout the remaining portion of the upper lobe and in patches throughout the lower lobe. In the right lung old tuberculous foci were found in the upper portion and tuberculous nodules throughout the remainder of the organ. The liver had undergone fatty degeneration; the intestines, large and small, were extensively ulcerated; the ulcerations were especially numerous in the small intestine. Many of the mesenteric glands were enormously enlarged, caseous, and some of them softened. A few tubercle bacilli were found in smear preparations from the granulations in the lung, but none could be demonstrated in the caseous material from the mesenteric glands.

(4) *Culture G D, human.*—This culture was obtained from the spleen of a guinea pig which had been inoculated with a small fragment of a peritoneal tubercle from a man 19 years old. The man was admitted to the Government Hospital for the Insane on February 11, 1902, suffering with acute mania associated with generalized tuberculosis. The tuberculosis apparently began about three months before admission to the hospital. The temperature ranged from 99° to 101.8° F. The sputum contained a very small number of tubercle bacilli. The patient gradually lost strength after admission, and died on May 7, 1902.

The autopsy revealed the following lesions: The lungs were adherent to the thoracic wall, except at the lower part of the left pleural cavity, where there was evidence of an old tuberculous empyema. Many miliary tubercles were scattered throughout both lungs but very few cavities were found. The bronchial glands were enlarged and caseous. The omentum, intestine, and all abdominal viscera were so bound together by inflammatory adhesions that it was impossible to remove any of the organs. The surface of the peritoneum was covered with yellowish white tuberculous masses ranging in size from a pin's head to one-fourth of an inch in diameter. The liver contained numerous tuberculous areas, some of which had broken down and produced cavities. The spleen was full of large tubercles; the kidneys showed numerous medium-sized tubercles. The intestines were matted together by adhesions and their mucous surface presented numerous small ulcers. There was also a tuberculous inflammation of the meninges of the brain. From the character of the disease it was impossible to determine accurately the mode of infection; it appeared probable, however, that the bronchial glands were the first of the internal organs to be involved, and it is not unlikely that the infection entered through the mouth or throat.

(5) *Culture No. 12,124, human.*—This culture was obtained from the spleen of a guinea pig which had been inoculated with a small fragment of the lung of a woman about 27 years of age. The woman was admitted to the Government Hospital for the Insane in November, 1900. She was an Indian, and was suffering from a form of congenital imbecility. She had lived the greater portion of her life in the woods, subsisting upon berries, game, and food which was voluntarily given to her. No detailed family history was obtainable. At the time of admission there was no suspicion of tuberculosis, but she began to fail shortly afterwards. She died on June 26, 1902.

The following synopsis of the postmortem lesions was kindly furnished us by Doctor Blackburn:

1. Tuberculous pleuritis, nodular, left side only. 2. Pulmonary tuberculosis with perforation. 3. Pneumothorax and pulmonary collapse. 4. Tuberculous ulceration of the intes-

tines. 5. Tuberculous lymphadenitis; mesenteric glands. 6. Tuberculous salpingitis with adhesions. 7. Perforation and localized peritonitis due to ulceration and adhesions of bowel in pelvic cavity. 8. Fatty infiltration of liver. 9. Moderate degree of interstitial nephritis. 10. Slight chronic lepto-meningitis and atrophy of brain. 11. Dilatation of left side of heart and cardiac thrombosis.

Remarks: The brief synopsis given shows the extent of distribution of the tuberculous lesions. The pulmonary disease seems to be ordinary acute phthisis. The pleural disease was unusual in that it was nodular and accompanied by active inflammation and was limited to one pleural cavity, the left. The nodules bore a considerable degree of resemblance to the pearl disease of cattle, a condition I do not often find. The intestinal ulcers were large and completely encircled the bowel. The primary seat of infection was probably the lungs.

(6) *Culture C No. 1, human.*—This culture was obtained from the spleen of a guinea pig which had been inoculated with a small fragment of a mesenteric gland from a child 19 months old. This child was admitted to the Children's Hospital on November 20, 1901. No family history could be obtained. The general history of the patient is as follows: A male child, fed partly at the breast and partly on cow's milk; had never enjoyed good health. On examination after admission to the hospital he was found to be markedly rachitic, and had a dull, apathetic expression. He was affected with a loose cough and his respiration was rapid and shallow. Mucous râles could be heard throughout the chest and some consolidation at the apex of the left lung was detected. The gastric and intestinal digestion was poor, but a physical examination of the abdomen failed to reveal anything abnormal. The patient gradually lost in condition from the time of his admission; appetite was and remained very poor, the pulse was rapid and weak, and he gradually passed into a moribund condition and died on December 22, 1901.

The autopsy record, which was kindly furnished us by Doctor Grasty, is as follows, the portions which have no direct bearing on tuberculosis being omitted:

Thorax: The diaphragm was adherent to the base of the right lung. There was a general adhesive pleurisy on this side with imperfect crepitation of the lung, which was mottled and grayish and studded with miliary tubercles which were especially numerous over the superior lobe and in the region of the apex. A cavity in the apex was about the size of a hazelnut. The left lung was consolidated at the apex. There was imperfect crepitation throughout the organ, and miliary tubercles from 1 to 5 mm. in size could be seen throughout the lung tissue. The lower lobe contained two large cavities filled with purulent material and threads of necrotic tissue. A microscopic examination of the contents of these cavities revealed the presence of many tubercle bacilli. The tracheobronchial glands were enlarged and caseous. Both pleuræ were studded with tubercles. The brain was congested and at its base some tubercles and a serous exudate were found. There were also tubercles in the cerebellum and fourth ventricle. In the abdominal cavity the liver was enlarged and mottled but showed no signs of tuberculosis. The spleen was enlarged and infiltrated with tubercles. The intestine was generally hyperemic. In the duodenum, the jejunum, and the ileum, at intervals of from 3 to 6 inches, were a number of ulcerations, some flat and broad, others deep, punched out, and undermined, all ulcers running transverse to the axis of the intestine. Scrapings from the ulcers revealed tubercle bacilli. In the caput coli there were two large ulcerated areas, one just above the ileocecal valve, 1½ inches in length and one-half inch wide and the other just at the ileocecal valve, 1 inch long and very irregular in outline. A number of tuberculous ulcers were found in the colon also. Many of the mesenteric glands were enlarged and caseous.

Guinea pigs were inoculated from the spleen and from the mesenteric glands, and all died of tuberculosis.

(7) *Culture D T, human.*—This culture was obtained from the spleen of a guinea pig which had been inoculated with a small fragment of the lung ot a girl 5 years old. The girl was admitted to the Children's Hospital February 28, 1902. The family history was negative. The child was reported to have been ill for approximately three months. On admission to the hospital her temperature ranged from 101° to 107° F. The clinical symptoms warranted a diagnosis of tubercular meningitis associated with pulmonary tuberculosis. The child died on March 10, 1902.

The autopsy was briefly as follows:

Some exudate at the base of the brain; the heart was normal; in the right lung there were a few tubercles. Hemorrhagic areas and tubercles were also found in the upper and lower lobes of the left lung. The liver was softened in places and old adhesions were found on its superior surface. The spleen was soft and exhibited old adhesions. The mesenteric glands were normal, as were the intestines and kidneys. There is nothing in the autopsy record to indicate infection by way of the digestive tract. Smear preparations from one of the lung tubercles showed a few tubercle bacilli.

(8) *Culture E T, human.*—This culture was obtained from the spleen of a guinea pig which was inoculated with a small fragment of a mesenteric gland from a girl 12 years old. The girl had been treated twice in the Children's Hospital for tubercular peritonitis and apparently cured. The first attack occurred during the winter of 1900 and the second in March, 1901. On July 6, 1901, she returned to the hospital suffering from a tuberculous arthritis of the elbow joint. In December, 1901, a complete excision of the joint was made and the child progressed well after the operation. On February 22, 1902, a cold abscess on the back of the neck was opened. On March 8, she began to complain of slight abdominal pain; the temperature rose gradually and the vomiting which occurred was followed rapidly by stupor which deepened into coma and the child died on the morning of March 9. The temperature shortly before death was 108.8° F. There was nothing in the history of this child to indicate the mode of infection.

The autopsy report, which was furnished us by Doctor Mason, is as follows:

Pleuritic adhesions on both sides. The lungs were free from tuberculosis; the heart was small and flabby. The abdominal cavity contained considerable fluid mixed with blood, pus, and intestinal contents. The omentum was much thickened and contained a large number of small tubercles. The omentum and intestines were adherent to the parietal peritoneum. The spleen was large and filled with tubercles; the kidneys were large and soft, but not tuberculous. The liver was very friable and filled with tubercles. The intestines were bound to the peritoneum and the neighboring coils were firmly adherent to each other. There were numerous caseous mesenteric glands; numerous ulcers distributed throughout the ileum. The lower portion of this section of the small intestine was constricted by bands of adhesive tissue and an area of about 7 inches was greatly congested and almost gangrenous. Just above this congested area were two perforations through small ulcers.

Smear preparations made from the mesenteric glands did not show any tubercle bacilli, but the guinea pigs which were inoculated with the same material developed the disease.

(9) *Culture C No. 4, human.*—This culture was obtained from the spleen of a guinea pig which had been inoculated with a small fragment of a peritoneal tubercle from a boy 5 years old. This child was admitted to the Children's Hospital on April 4, 1902. The family history and previous history of the patient as determined at the hospital were as follows: The parents were both alive and healthy, and had lost no children. The only other child was in good health. There was a history of some tuberculosis in the mother's family. The boy when an infant had been reared on condensed milk and until the time of his present illness was said to have been a healthy child. During the summer and fall of 1901 ascites developed and the child was confined to bed for two weeks, the effusion disappearing, but a hard mass remained in the region of the umbilicus. During October, 1901, the patient developed pertussis, and the cough persisted until the time of admission to the hospital. About two weeks before entering the hospital, during coughing, a rupture of the abdominal wall occurred and an intestinal fistula resulted. When admitted to the hospital the child was extremely emaciated, had a severe cough, fever at night, seemed constantly disposed to sleep, and had no appetite. The patient did not improve in the hospital, and died six days after admission.

The autopsy was briefly as follows:

There was a generalized tuberculosis of the thoracic and abdominal viscera involving the lungs, spleen, liver, intestines, and lymph glands. The peritoneum and mesentery contained many tubercles. The mesenteric glands were generally caseous and enlarged. In the lower portion of the ileum was a perforation which connected with a cavity (completely walled off from the peritoneal cavity) which communicated with an opening at the site of the umbilicus. The intestines were so firmly bound down by adhesive tissue that it was exceedingly difficult to trace their course. No ulcers were found. The peritoneal tubercles were examined microscopically but no bacilli found. The guinea pigs which were inoculated developed tuberculosis. It seems probable that the infection in this case as well as in the preceding one, had its origin in the abdominal cavity, but from the rather meager details of the autopsy which are obtainable it is impossible to speak definitely in regard to this matter. On account of the age of the child and its previous good health, the infection most probably took place at some time subsequent to the date when it was weaned from its bottle.

(10) *Cultures Nos. 49 to 60.*—These cultures were of human origin. They had been grown in the laboratory for a number of years on liquid media and still retained the characteristic virulence for guinea pigs.

(11) *Cultures A 101 to A 125.*—These cultures represent different generations of the attenuated culture which has been under cultivation in the Biochemic Division for about ten years. Culture A 125 is the one hundred and fiftieth generation on glycerinized bouillon. This culture was originally received from Doctor Trudeau, who states that it was obtained by himself in 1891 directly from a case of miliary tuberculosis in man. It was afterwards passed through a rabbit and then cultivated on various artificial media. While entirely avirulent for guinea pigs, it has the property of producing in cultures the substance which causes the fever reaction in tuberculous animals, and has for a long time proved very satisfactory for the preparation of tuberculin.

(12) *New York culture, human.*—This culture was obtained December 21, 1901, from a tuberculous lung which was sent to us by Doctor Lartigau, of the College of Physicians and Surgeons in New York. Similar material was also secured from New York on January 6, February 10, and March 3, 1902.

(13) *Sputum, human.*—This material was used for the inoculation and drenching of cattle.

SOURCES OF BOVINE TUBERCULOUS MATERIAL.

(14) *Culture bovine III* (Smith).—This was given us several years ago by Dr. Theobald Smith and since then has been cultivated continuously on glycerinized bouillon.

(15) *Culture C No. 81, bovine.*—This culture was obtained from the spleen of a guinea pig which had been previously inoculated with a fragment of a mediastinal gland from cow No. 81. This animal—cow No. 81—was affected with tuberculosis of spontaneous origin.

Record of cow No. 81: About 5 years old; June 25, 1901, tested with tuberculin; reacted; was removed from the healthy animals and placed in a stable with a number of other cattle which had also reacted to tuberculin; August 26, 1901, had a moist, dry cough, respiration regular and not labored; January 2, 1902, was removed to another stable and confined continuously in a box stall adjoining stalls in which other tuberculous animals were kept. Killed March 13, 1902, and examined postmortem.

Autopsy: Right lung is a solid mass of tubercles excepting a small area at the superior posterior portion of principal lobe. Left lung contains many tuberculous areas, but is not affected to so great an extent as the right one. The tuberculous masses, on section, are found to contain lime salts, and many are broken down. There are also a number of distinct cavities which are partially filled with a gelatinous material. These cavities communicate with the bronchi, which, as well as the trachea, contain a large amount of the above material. The mediastinal glands are enlarged and are converted into masses of tubercles separated from each other by thickened connective tissue. These tubercles, on section, are found to consist of a firm, cheesy material, which contains lime salts. Liver contains several characteristic tuberculous nodules. Spleen apparently normal. Nearly all of the mesenteric glands contain one or more tuberculous foci.

(16) *Bovine tuberculous tissue.*—Material obtained from various cases of spontaneous tuberculosis in cattle.

EXPERIMENTS UPON HOGS.

It will be remembered that Professor Koch[1][a] announced that he had been unable to produce generalized tuberculosis in hogs by subcutaneous inoculations with pure cultures of human origin. In order to determine the correctness of his views we inoculated ten hogs—some with human tuberculous material and some with pure cultures of the human tubercle bacillus—and also one pig with a bovine culture, the latter animal serving as a comparison with those inoculated with human virus. As will be seen from the gross appearance of the various organs obtained from some of these animals, there is little or no difference between the animals inoculated with bovine and human tuberculous material (Pls. VI, VII, VIII, human, and X, XI, XII, bovine). In the case of one pig no disease was produced by inoculation with a human culture. This culture proved to be quite low in virulence for all of the domesticated animals which were exposed to it. The detailed records of the hogs follow:

Record of hog No. 230.—January 29, 1902, tested with tuberculin, without reaction. April 19, 1902, weight 57¼ pounds. Injected subcutaneously inside of right thigh with 1½ c. c. of a suspension of human culture C No. 1 prepared in the following manner: The colonies of tubercle bacilli were scraped from the surface of four dog-serum culture tubes and a watery suspension of them made by adding 18 c. c. of sterile water. A portion of this suspension, which was moderately turbid, was used to inject the animal. September 9, 1902, killed and examined postmortem.

Autopsy: In good condition; no lesions of tuberculosis.

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[a] These figures refer to bibliography on page 98.

Record of hog No. 226.—April 12, 1902, weight before injection, 51 pounds; tested with tuberculin before inoculation without reaction; a piece of thickened tuberculous human intestine, same source as human culture C No. 4, introduced beneath the skin inside of the left thigh; the usual technique employed. September 9, 1902, the animal killed and examined postmortem.

Autopsy: The entire lung was sprinkled with areas of tuberculosis; the left median lobe completely solidified; the bronchial and mediastinal glands greatly enlarged and entirely tuberculous; portal glands similarly affected; a number of necrotic foci in the liver.

Guinea pigs Nos. 2300 and 2301 were each inoculated subcutaneously with a small fragment of a mediastinal gland from this hog and as a result died of a generalized tuberculosis.

Record of hog No. 229.—April 12, 1902, weight 58 pounds; tested with tuberculin before inoculation without reaction; a piece of thickened tuberculous intestine introduced beneath the skin on the inside of the left thigh; the usual technique employed; same material as that used for hog No. 226. September 9, 1902, killed; in good condition.

Autopsy: Sprinkled throughout the lung are numerous minute white points surrounded by a zone of semitransparent material. The mediastinal glands show a number of minute necrotic areas and are slightly enlarged; liver sprinkled with small areas of necrosis, and the portal glands are enlarged and filled with necrotic material; more or less disease of the omentum and serous surface of the intestines, resembling pearl disease; several necrotic areas in the spleen.

The tissue used to inoculate hogs Nos. 226 and 229 was from the same source as that used for obtaining the culture with which hog No. 460 was inoculated.

Record of hog No. 460.—This animal was not tested with tuberculin before inoculation, but was in a perfectly healthy condition at that time. October 13, 1902, injected subcutaneously inside of left thigh with 2 c. c. of human culture C No. 4. This animal showed signs of illness on October 30. The disease advanced quite rapidly, the animal exhibiting a cough, labored respiration, and a poor appetite until November 11, when it was in a dying condition and was killed.

DESCRIPTION OF PLATE IX.

Sections of organs of hogs inoculated subcutaneously with cultures C No. 4 and E T, human, tuberculosis. Stained with hematoxylin and carbol-fuchsin. Magnified 1,450 diameters.

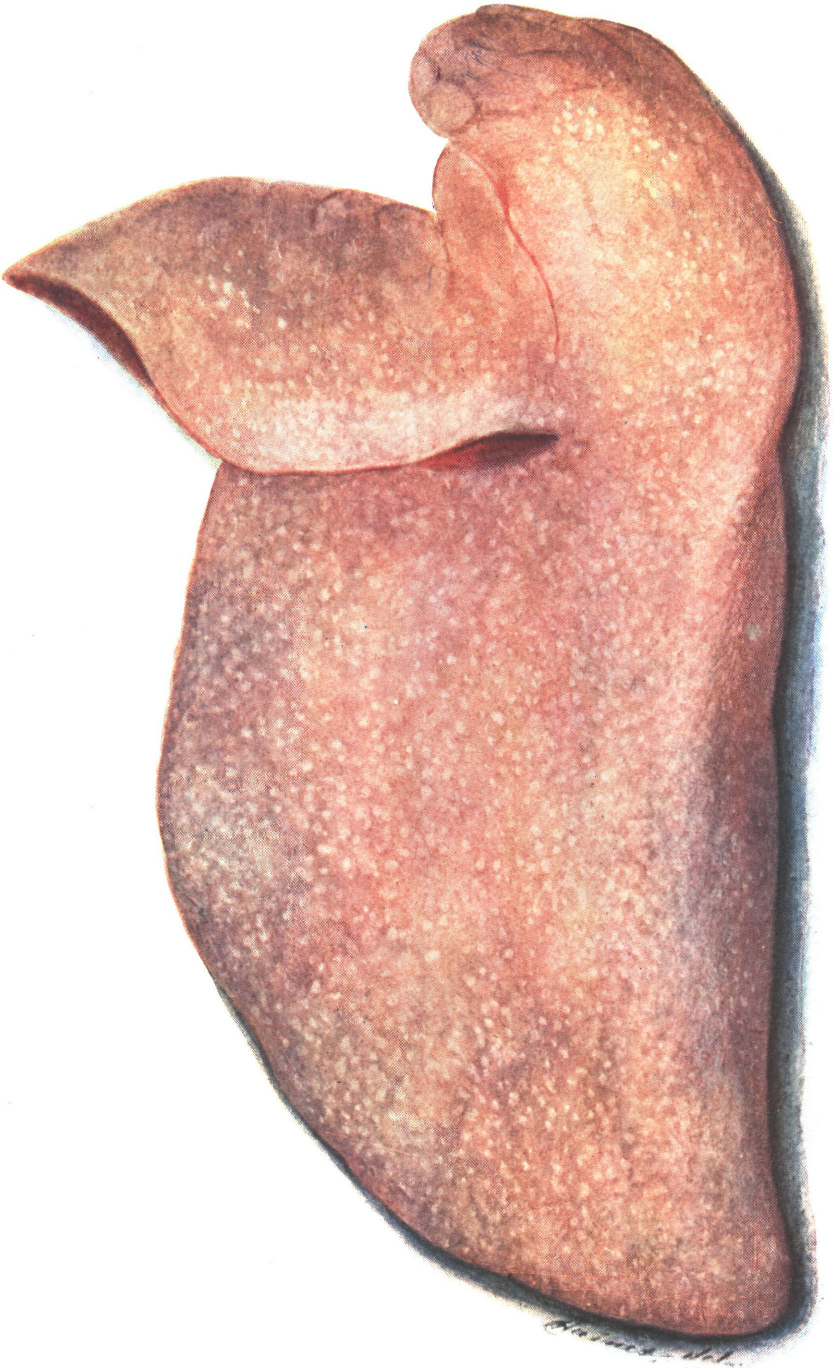
Fig. 1.—Section of lung of hog No. 457, inoculated with culture E T.

Fig. 2.—Section of spleen of hog No. 457, inoculated with culture E T.

Fig. 3.—Section of liver of hog No. 457, inoculated with culture E T.

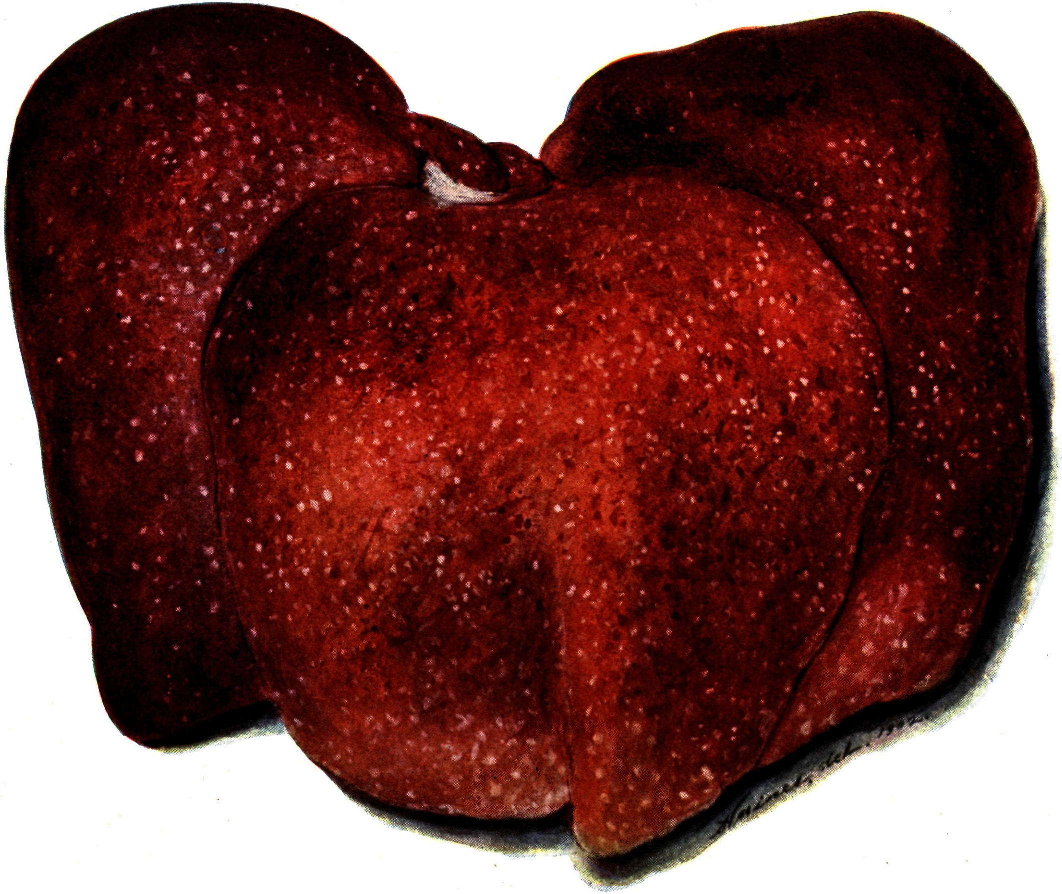
Fig. 4.—Section of lung of hog No. 460, inoculated with culture C No. 4.

Drawing by W. S. D. Haines.

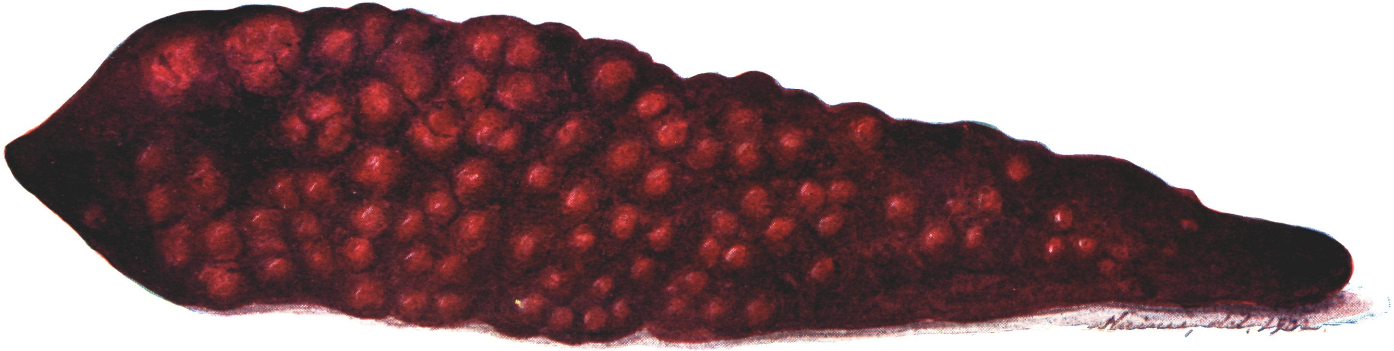


Lung hog 460. Subcutaneous inoculation with pure culture human tuberculosis No. C 4.

Plate VII.



Liver hog 460. Subcutaneous inoculation with pure culture human tuberculosis No. C 4.



Spleen hog 457. Subcutaneous inoculation with pure culture human tuberculosis No. E. T.

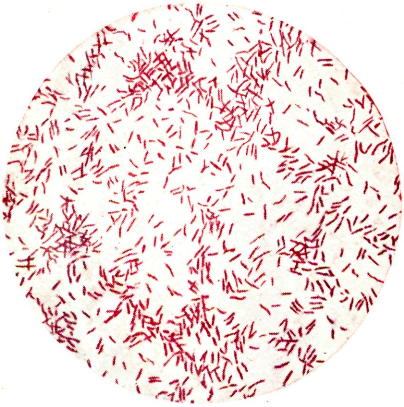


Fig. 1.

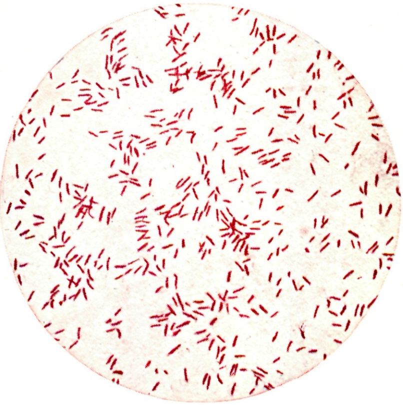


Fig. 2.

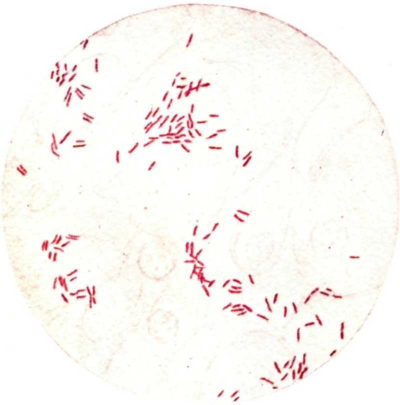


Fig. 3.

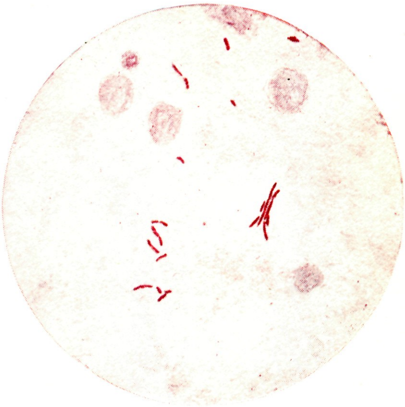


Fig. 4.

Tubercle bacilli in Sections from hogs. Human tuberculosis.

Autopsy: Some swelling and slight necrosis of tissue on the inner side of the left thigh was noted. Lymphatic system: (1) Left inguinal glands much enlarged, containing many small yellowish nodules visible on surface of the glands; (2) right inguinal gland and (3) right lumbar gland apparently normal; (4) retroperitoneal glands enlarged; (5) posterior mediastinal glands enlarged, size of pigeon's egg. Spleen very dark red in color; the surface is quite rough and nodular; there is, however, no sign of caseation in the nodules. Liver is paler than normal, and scattered thickly over its surface are grayish white areas, fairly well defined and about the size of a pinhead. Kidneys rather dark, but otherwise normal. Intestines not inflamed, but mucous surface of cecum is covered with a thick white exudate, which is easily peeled off, leaving the intestine beneath in a fairly healthy condition. Lungs have a general pearl-gray color and scattered over their entire surface numerous very minute whitish areas can be distinguished. (See Pls. VI and VII.)

Microscopical examination: Lung—A number of well-defined tubercles with necrotic centers; the tubercles contain a fair number of tubercle bacilli. Liver—A large number of miliary tubercles with necrotic center; occasionally a giant cell can be seen; a few tubercle bacilli are present in some of the tubercles. Spleen—A number of more or less circumscribed areas of necrosis are visible; these areas contain a very few tubercle bacilli. Mediastinal glands—There is considerable necrosis of the cellular tissue and a very few tubercle bacilli are present. Mesenteric gland—A few necrotic areas and a few tubercle bacilli were noted. Left inguinal gland—Large areas of necrosis which contain a few tubercle bacilli. Left lumbar gland—Large areas of necrosis containing very numerous tubercle bacilli. Intestinal exudate—No tubercle bacilli were found.

Record of hog No. 459.—Tested with tuberculin before inoculation without reaction. January 24, 1903, weight 40 pounds; injected subcutaneously inside of left thigh with 2 c. c. of a thin suspension of human culture C No. 4. This animal appeared to be in good condition until June 2, 1903, when it was killed and examined postmortem, lesions of generalized tuberculosis being found.

Record of hog No. 457.—This animal was not tested with tuberculin prior to inoculation, but was in a perfectly healthy condition on October 13, 1902, when it was injected subcutaneously inside of left thigh with 2 c. c. of a thin suspension of human culture E T. This animal gradually lost in condition and died on December 13, 1902.

Autopsy: There was some swelling at seat of inoculation. Lymphatic system—(1) Left inguinal glands very much enlarged and firm; (2) right inguinal glands normal in size and appearance upon section; (3) right lumbar glands apparently normal; (4) retroperitoneal glands enlarged and tuberculous; (5) posterior mediastinal glands very slightly enlarged. Abdominal viscera—(1) Liver about normal size, mottled, and rather light in color; (2) spleen about normal in size, covered with light-red nodules about size of a half pea, some smaller, which are raised above the surface of the organ—a typical tuberculous hog spleen (see Pl. VIII); (3) kidneys congested. Thoracic viscera—(1) Heart, slight pericardial effusion; (2) lungs both gray and almost completely solidified, a number of small grayish points over the surface.

Microscopic examination: The several organs showed as follows: Lung—Large areas are solidified and necrotic; many of the alveoli are filled with cellular débris in which are enormous numbers of tubercle bacilli. Liver—There are large numbers of minute tubercles in the section; these tubercles contain numerous tubercle bacilli. Spleen—A number of large, fairly-well defined tubercles with necrotic centers are to be noted; these tubercles contain great numbers of tubercle bacilli. Mediastinal gland—Contains a moderate number of tubercle bacilli. Left inguinal gland—Tubercle bacilli are present in enormous numbers. Left lumbar gland—Tubercle bacilli are found in enormous numbers.

Record of hog No. 455.—Tested with tuberculin before inoculation, without reaction. January 24, 1903, weight 44 pounds; injected subcutaneously inside of left thigh with 2½ c. c. of a thin suspension of human culture E T. This animal showed no especial signs of illness as a result of the inoculation, but when killed on June 2, 1903, exhibited the lesions of generalized tuberculosis.

Record of hog No. 483.—Tested with tuberculin before inoculation but did not react. January 24, 1903, weight 35½ pounds; injected subcutaneously inside of left thigh with 2 c. c. of a thin suspension of bovine culture C No. 81. March 3, 1903, losing in condition; respiration labored and accelerated; has a tumor at seat of injection about 2 by 2½ inches in size. This hog failed rapidly and died March 9, 1903.

Autopsy: Slight inflammation was found on inside of left thigh; left inguinal gland enlarged and caseous; right inguinal glands normal; left lumbar gland considerably enlarged; mediastinal glands somewhat enlarged. Spleen slightly enlarged and dotted with small round pale areas, some of which are slightly raised above the surface of the organ. Liver has scattered over its surface a fair number of small grayish areas the size of a pinhead or smaller. Lungs were both distended and did not collapse when the thoracic cavity was opened; there was a red hepatization of the cephalic lobes of both lungs; the principal lobes were generally grayish in color and were sprinkled with minute translucent gray areas. (See Pls. X, XI, and XII.)

TABLE III.—*Inoculation of hogs subcutaneously with human and bovine tuberculosis.*

Material used.	Dose. c. c.	Number of animal.	Date of inoculation.	Result of inoculation.
Human culture E T	2	Hog No. 457[a]	Oct. 13, 1902	Died Dec. 13, 1902.
Do	2.5	Hog No. 455[b]	Jan. 24, 1903	Remained in fair condition.
Human culture C No. 4	2	Hog No. 460[c]	Oct. 13, 1902	In dying condition when killed.
Do	2	Hog No. 459[b]	Jan. 24, 1903	Remained in fair condition.
Bovine culture C No. 81	2	Hog No. 483[a]	do	Died March 9, 1903.
Human culture C No. 1	1.5	Hog No. 230[d]	Apr. 19, 1902	Remained well.
Human intestine (tuberculous).	Small piece.	Hog No. 226[e]	Apr. 12, 1902	Remained in fair condition.
Do	do	Hog No. 229[e]	do	Remained fairly well.

The following footnotes show the condition of the hogs after death:

[a] Generalized tuberculosis.

[b] Killed June 2, 1903. Generalized tuberculosis.

[c] Killed Nov. 11, 1902. Generalized tuberculosis.

[d] Killed Sept. 9, 1902. No tuberculosis.

[e] Killed Sept. 9, 1902. Generalized tuberculosis.

DISCUSSION OF HOG EXPERIMENTS.

From the experiments which have just been described it will be seen that of three cultures of tuberculosis from children, one proved to be practically without virulence for hogs, while the other two induced a generalized tuberculosis which was quite as severe as the disease caused by the bovine bacillus in hog No. 483. The gross lesions brought about in the hogs by the more virulent human cultures and the bovine culture are very well depicted in the accompanying plates (VI to XII).

All the hogs used for these inoculations were in a perfect state of health at the beginning of the experiments.

As a result of these experiments upon hogs, the conclusion is justified that—

1. Certain tubercle bacilli of human origin possess quite as great pathogenic power for hogs as tubercle bacilli of bovine origin.
2. The disease induced in hogs by human cultures, C No. 4 and E T, was distinctly progressive in character, death taking place in two of the animals in twenty-eight and sixty days, respectively.

EXPERIMENTS UPON CATTLE.

Every precaution was taken to avoid the possibility of error in these experiments. In all cases perfectly healthy animals were selected, and no cattle were included in the experiments that had not failed to respond to the tuberculin test. Each animal was kept, after inoculation, in a clean, separate stall, where there was no opportunity for the contraction of disease from other animals. The cattle were inoculated subcutaneously, intraabdominally or intravenously, and some were fed or drenched with tuberculous material. In some instances it was possible to inoculate or drench cattle with human tuberculous tissue and later to inoculate others with pure cultures derived from the same human tissue. By this method we hoped to determine whether the virulence of the organism in question was heightened or lowered during the process of its isolation in pure culture. The dose given the cattle varied somewhat, but was in the main fairly uniform and not excessive, as will be seen by the examination of the records which follow.

INTRAVENOUS INOCULATIONS

Record of heifer calf No. 270.—About one year old; tested with tuberculin without reaction. September 16, 1902, injected with 5 c. c. of a suspension of human tubercle culture F T in right jugular vein. The first symptom of illness was noted on September 25, when the respiration was accelerated. The respiratory disturbance became gradually more marked and the general condition of the animal became worse until about the 17th of October, 1902, at which time signs of improvement were noted. The animal continued to gain rapidly in condition and appetite, and the respiration became normal.

On January 24, 1903, the animal, apparently in perfect health, was inoculated subcutaneously in front of the right shoulder with 5 c. c. of bovine III tubercle culture. As a result of the inoculation with the bovine virus a tumor, 2 by 3 inches in size, developed at the point of inoculation, and the neighboring prescapular gland became considerably enlarged. On May 19, 1903, a soft tumor of considerable size was visible at the point of inoculation; the prescapular gland had returned to its normal size. The enlargement on the side of the neck began to discharge a thick creamy pus on July 27, 1903. On October 24, 1903, a tuberculin test of this animal gave no reaction. The heifer was killed on November 2, 1903, and examined postmortem.

Autopsy: The autopsy showed no lesions of disease. The human culture had evidently not only failed to infect this animal but, on the contrary, apparently had produced an immunity, although such a supposition is open to the rather serious objection that there was no control to prove the pathogenic power of this particular bovine culture for normal cattle.

Record of calf No. 275.—Age, about 9 months; tested with tuberculin previous to inoculation, but did not react. September 16, 1902, injected with 5 c. c. of a suspension of human culture F L into right jugular vein; weight at time of inoculation, 186 pounds. On September 27 the animal was losing in condition and its respiration was accelerated. This calf became gradually weaker until November 3, 1902, when it was killed and examined postmortem.

Autopsy: The pleuræ were considerably thickened and opaque; the lungs showed numerous very minute foci of disease, and the same was true of the liver and kidneys. The mediastinal glands were enlarged, congested, and had the appearance of beginning tuberculosis.

Microscopic examination: Lung—Most of the air spaces in the section are filled with desquamated epithelial cells and leucocytes; there is a slight general increase in connective tissue throughout section; no areas of necrosis were found, but numerous long, well-stained, beaded tubercle bacilli were visible. Kidney—Rather severe cloudy swelling; no distinct tubercles found, but a few long faintly stained tubercle bacilli could be seen. Mediastinal gland—A number of ill-defined areas of coagulation necrosis and a very few long, slender, faintly stained tubercle bacilli.

Although this animal was made quite sick by the intravenous injection of culture F L, the tuberculous lesions found at the autopsy were not progressive in character, and seemed to be hardly greater than might have been expected from the mechanical irritation and the toxic action of the bodies of the injected bacilli. We must consider this case, therefore, as one in which human bacilli failed to produce a true tuberculosis in cattle after intravenous inoculation.

Record of calf No. 272.—About 8 months old; weight, 244 pounds. September 10, 1902, tested with tuberculin, without reaction. September 15, 1902, injected 5 c. c. of human tubercle culture S E No. 3 into right jugular vein. As a result of this inoculation the animal exhibited considerable disturbance of respiration, but did not lose very much in condition.

On December 8, 1902, the calf appeared to be again in a perfect state of health, and having remained well, it was inoculated subcutaneously in front of right shoulder with 5 c. c. of a suspension of bovine tubercle culture III on January 24, 1903. This inoculation of bovine culture produced an extensive local swelling which, in course of time, softened and discharged a thick, creamy pus; the general condition of the animal, however, remained good. On November 6, 1903, this calf was killed and examined postmortem.

Autopsy: The lungs contained a number (50 to 75) of small nodules, which varied in size from 1 to 5 mm. These nodules had a pearl-gray glistening appearance, and in a few instances were surrounded by a zone of congestion. The epicardium showed a little roughening along the groove between the two ventricles. At the seat of the inoculation with the bovine culture the subcutaneous tissues were greatly indurated and sprinkled with several tubercular nodules, which contained a soft, creamy pus. The lymph gland near this point was greatly enlarged, but showed only one nodule of tuberculosis, about 1½ cm. in diameter, which contained thick, creamy pus. Guinea pigs inoculated with fragments of a minute nodule in the lung did not contract tuberculosis.

It is difficult to say positively whether the pulmonary nodules were referable to the intravenous inoculation with human culture S E No. 3 or whether they were caused by the later subcutaneous inoculation with bovine culture III. It seems probable, however, in view of the fact that the bovine virus did not affect the deeper lymph glands, that the nodules in the lungs were caused by the injection of the human tubercle culture. This pulmonary disease was, however, not progressive; the pathological changes were very slight, and apparently did not interfere with the health of the animal, notwithstanding the fact that fourteen months had elapsed between the date of inoculation and the autopsy. On this account we are inclined to consider this inoculation negative in so far as the production of tuberculosis by culture S E No. 3 is concerned.

Record of calf No. 271.—Age, about 8 months; weight, 226½ pounds. September 10, 1902, tested with tuberculin without reaction. September 16, 1902, injected 5 c. c. of human tubercle culture G D into right jugular vein. This animal began to show symptoms of a respiratory disturbance on September 27. This continued for some time without very great effect upon the general health of the calf. On November 4 a rather severe conjunctivitis was noted. This was accompanied by a profuse serous discharge from the eyes. This inflammation did not improve, but, on the contrary, became continually worse. In addition, the animal suffered from a severe diarrhea, which began on November 6 and continued until its death. On November 26 the calf, being in a helpless condition, was killed.

Autopsy: No gross lesions were found, with the exception that the mediastinal glands contained several necrotic foci, the eyeballs were coated with a semiopaque membrane, and the mucosa of the fourth stomach was sprinkled with many elevations. These elevations were from one-eighth to three-sixteenths inch in diameter, and had depressed centers. Liver, spleen, and kidney normal. Lung mottled; some areas of consolidation. Mediastinal glands, rough and nodular. Fourth stomach—The mucous surfaces show some very small white ulcers, and attached to the peritoneal surface are a few nodules the size of a pea.

Microscopic examination: Lung—Numerous slender, beaded tubercle bacilli were found in smear preparations from this organ. In the nodules on peritoneal surface of fourth stomach a moderate number of long, slender, beaded tubercle bacilli were found. In smear preparations from a small ulcer on mucous surface of fourth stomach a few slender, beaded bacilli were found.

In the absence of intestinal lesions of tuberculosis, the severe diarrhea from which this animal suffered can hardly be ascribed to that cause, and as the poor condition of the animal was evidently due directly to the diarrhea, it must be granted that death was not due to the inoculation with the human tubercle bacillus. It is of interest to note, however, that two months after the inoculation macroscopic lesions of tuberculosis, together with tubercle bacilli, were present in the lung and on both the serous and mucous surfaces of the fourth stomach. On the mucous surface of the fourth stomach there was some destruction of tissue.

Record of calf No. 289.—About six months old. October 11, 1902, tested with tuberculin without reaction. October 14, 1902, inoculated intravenously with 5 c. c. of human tubercle culture 12124.

The history of this calf is quite similar to that of calf No. 271. The inoculation with tubercle bacilli produced an accelerated respiration after a time, but the health of the animal did not seem to be seriously impaired until December 20, when an inflammation of the eyes was noted. This affection of the eyes became gradually worse, and was accompanied by a severe diarrhea, the animal losing continually in condition. On February 12, 1903, it was killed. At the autopsy the lungs were found to be sprinkled with a considerable number of small tumors, probably due to the action of tubercle bacilli. This case must be viewed as another failure to produce a progressive tuberculosis in calves by the intravenous injection of human tubercle bacilli, although localized changes were produced in the lungs.

Record of calf No. 273.—About 8 months old; weight 240 pounds. September 10, 1902, tested with tuberculin without reaction. September 15, 1902, injected 5 c. c. of a suspension of human tubercle culture C No. 1 into right jugular vein. The animal exhibited no ill effects as a result of the injection.

On January 24, 1903, it was injected subcutaneously in front of right shoulder with 5 c. c. of a suspension of bovine tubercle culture III. This injection produced quite a large local swelling after a time, and the adjacent prescapular gland became considerably enlarged; the general condition of the animal remained good. On May 18 the local swelling had diminished and the prescapular gland had returned to its normal size. The general condition of the animal remained good, although the tumor at the seat of injection persisted until October 24, 1903, when the calf was tested with tuberculin and a reaction obtained. The animal was killed on November 13, 1903, and examined postmortem.

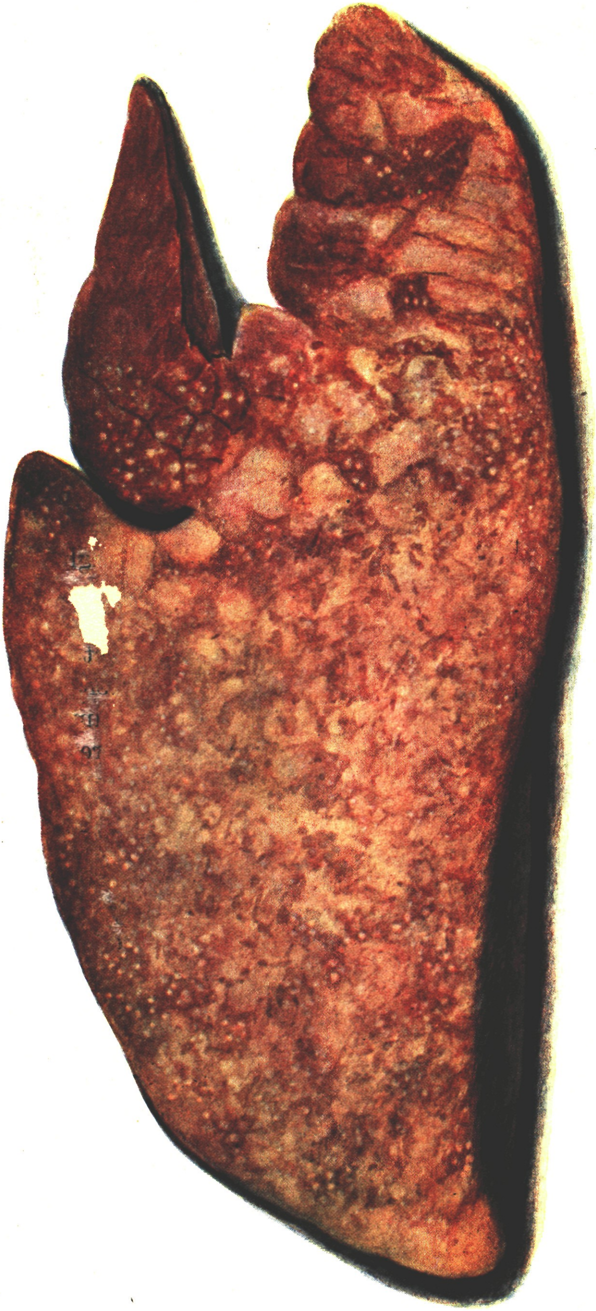
Autopsy: General condition good. A tumor about 2 inches long and 1 inch thick, consisting of a thin wall inclosing a mass of thick, soft pus was found at the seat of injection on the right side. The gland adjacent to the injection tumor is greatly enlarged and contains several tubercles 5 mm. in diameter. Other superficial glands are normal. Lung—Mediastinal glands are considerably enlarged, but free from disease; sprinkled over the pleura of the lung are numerous tubercles from 1 mm. to 5 cm. in diameter; a few small tubercles in the lung tissue proper; the number of tubercles on the costal pleura and on both sides of the diaphragm. Liver—Several tubercular masses on the surface of the liver from 1 to 3 cm. in diameter. Other organs normal.

Owing to the absence of any symptoms of illness following the inoculation with the human culture, the lesions found at the autopsy must be attributed, at least in great part, to the action of the bovine bacilli. This calf did not, therefore, acquire immunity from the bovine bacillus as a result of the previous intravenous injection with the human culture.

Record of calf No. 264.—Age about 5 months. Tested with tuberculin, but did not react. August 9, 1902, injected 5½ c. c. of a suspension of human tubercle culture E T into jugular vein. No symptoms of illness were noted until August 19, when the respiration was accelerated and the temperature high. From this date until the day of its death the animal became rapidly worse. There was loss of appetite, listlessness, emaciation, and an uncertain gait. The respiration was short and jerky, with an occasional cough. On August 29, 1902, twenty days after injection, the calf died and was examined postmortem.

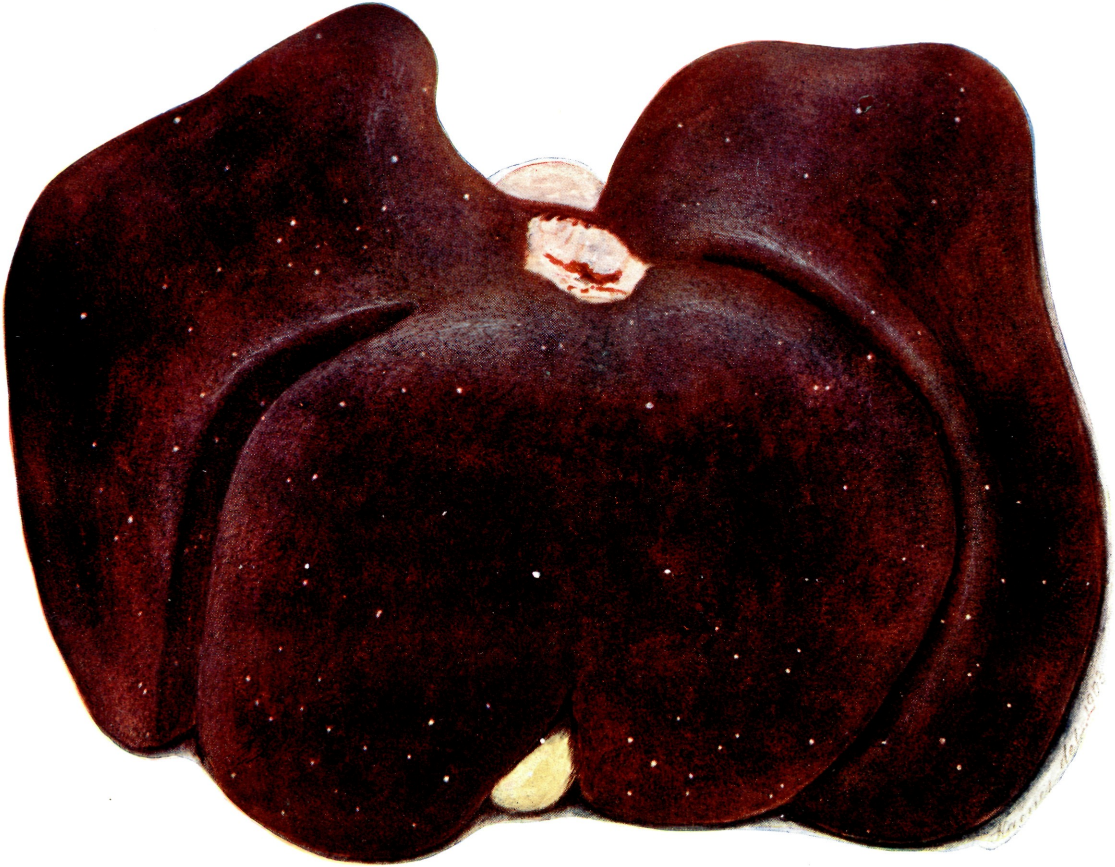
Autopsy: Animal greatly emaciated. Mediastinal glands greatly enlarged, edematous, and congested. Lung intensely congested; the anterior lobes are in a state of collapse, and show numerous hemorrhages under the pleura; sprinkled over the surface are numerous minute necrotic areas; on section the lung has a finely granular appearance, apparently a miliary tuberculosis of the entire organ; bronchial tubes show some slight congestion, and the smaller tubes contain a small amount of viscid mucus. Liver—Greatly swollen, edges rounded; the centers of the lobules appear as pale, yellowish gray points of irregular outline. Portal lymph glands pale but very edematous. Spleen—Enlarged and softened; trabeculæ and Malpighian corpuscles distinct. Kidneys—Redder than usual, and show a sprinkling of white areas, few in number. Mesenteric glands edematous and congested. The glands at the brim of pelvis and just inside of the anterior portion of thorax are apparently normal. Throat glands edematous.

Microscopic examination: Lung—The lung tissue examined in section was infiltrated with cells, and there were a number of miliary tubercles scattered throughout it, all of which contained numerous tubercle bacilli. Liver—No distinct tubercle formation was observed, but a number of well-stained tubercle bacilli were seen in various portions of the section, usually in small clumps. The liver showed rather extensive fatty changes. Kidneys—Marked parenchymatous degeneration; no tubercle formation, though a few tubercle

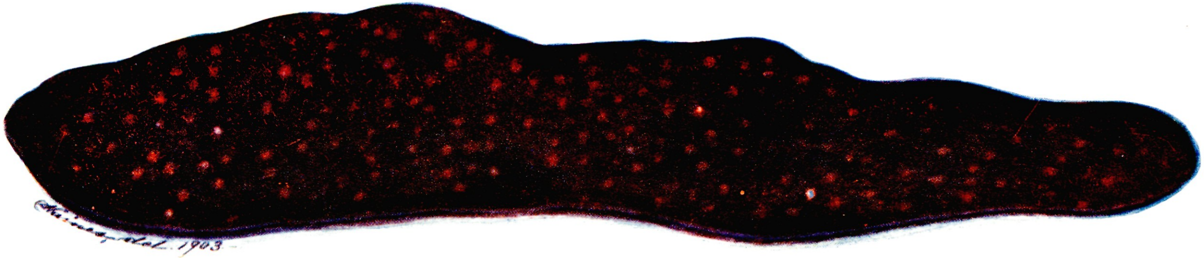


Lung hog 483. Subcutaneous inoculation with pure culture bovine tuberculosis No. 81.

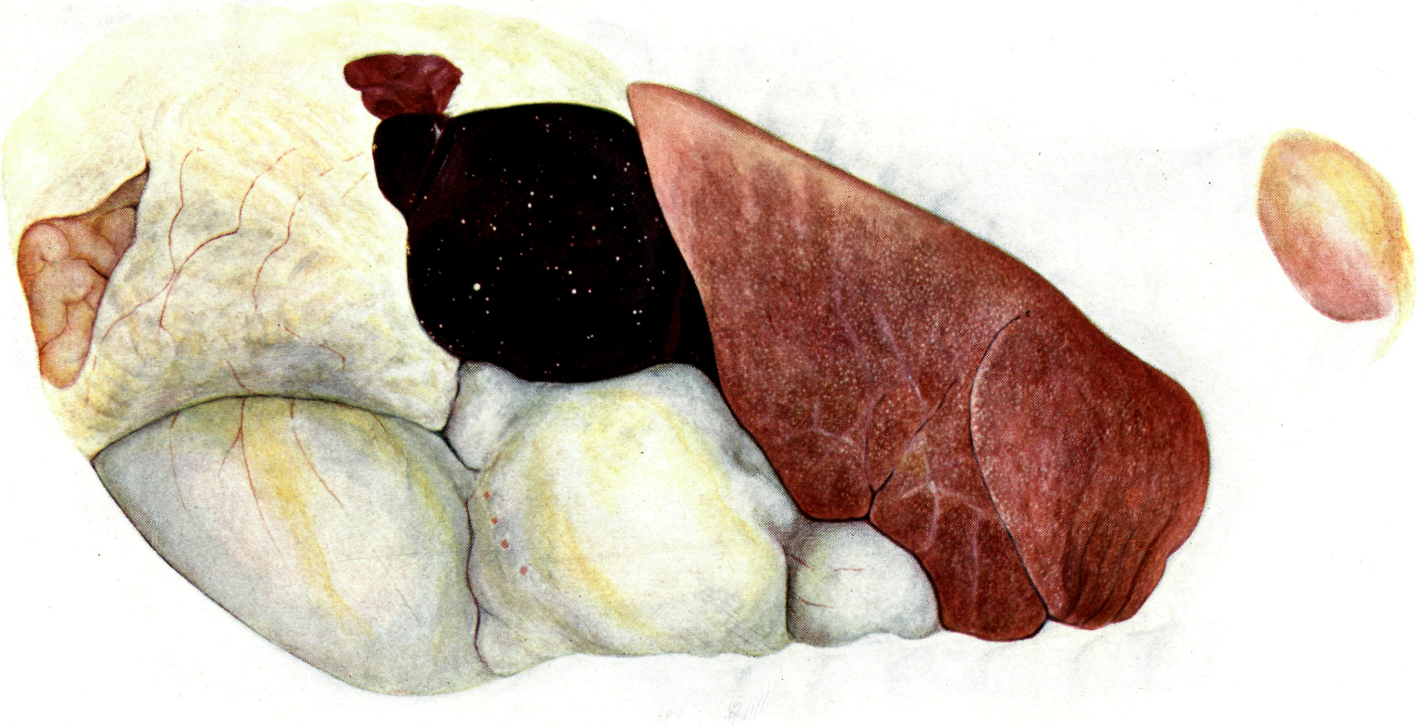
Plate XI.



Liver hog 483, subcutaneous inoculation with pure culture bovine tuberculosis No. 81.



Spleen hog 483. Subcutaneous inoculation with pure culture bovine tuberculosis No. 81.



Calf 286. Subcutaneous inoculation with pure culture human tuberculosis No. C4.

bacilli were found in smears. Spleen—No sharply defined tubercles were seen, although there were some rather large areas of coagulation necrosis in which were found some giant cells and a large number of tubercle bacilli. Mediastinal gland; a very large number of well-stained tubercle bacilli were seen throughout the section; in some places there was a beginning necrosis of the cellular tissue and here the tubercle bacilli were especially abundant. Mesenteric gland—The portions seen in section were apparently normal, although a very few tubercle bacilli were found in smears from this gland. A guinea pig inoculated with a bit of lung tissue from calf No. 264 died of generalized tuberculosis.

There can be no doubt as to the result of this experiment. The human culture used for intravenous injection produced a severe generalized tuberculosis and was evidently directly responsible for the death of the calf. It is instructive to compare this autopsy with that of calf No. 260, inoculated with bovine III culture.

Record of calf No. 265.—Age, about 5 months. Tested with tuberculin before inoculation without reaction. August 9, 1902, injected 5½ c. c. of a suspension of human tubercle culture D T into right jugular vein. Began to lose in condition on August 22, 1902, but did not seem to be seriously ill until August 30, when the respiration had become very much accelerated and the animal appeared to be failing rapidly. The respiration became more rapid and labored and the emaciation and weakness gradually more marked. The calf was found dead September 8, 1902, thirty days after injection.

Autopsy: Lung very much congested and edematous; on section showed evidences of extensive broncho-pneumonia; other organs in very much the same condition as described for calf No. 264.

Microscopic examination: Lung—Large areas are solidified; a few tubercle bacilli were found in smears. Liver has undergone extensive degenerative changes; no tubercle bacilli could be found, but great numbers of a large bacillus which did not stain by Gabbett's method were found in the blood vessels. Spleen—No tubercle bacilli could be found, but there were enormous numbers of bacilli of the kind found in liver. Kidney—No tubercle bacilli could be found; the sections showed extensive cloudy swelling and the blood vessels were filled with the same large organism which was found in liver and spleen. Mediastinal gland—Smears showed fairly numerous tubercle bacilli and in the section many of the large bacilli previously mentioned were seen. A guinea pig inoculated subcutaneously with a bit of lung from calf No. 265 died of generalized tuberculosis.

Although at the autopsy the lesions gave every indication that the animal died of a severe generalized tuberculosis quite similar to the disease to which calf No. 264 succumbed, we are led to doubt this as a result of the microscopic examination. No tubercle bacilli could be found in any of the organs with the exception of the lungs and mediastinal gland. In addition, the kidneys, spleen, and liver contained enormous numbers of an unidentified bacillus which was not acid fast. Under these circumstances the probability of a secondary infection seems very great, and the tuberculosis injection can not be considered as having produced, unaided, the lesions found at the autopsy. Unfortunately, this secondary infection was not suspected until the sections were examined, and we were therefore unable to study cultures of the organism associated with it.

Record of calf No. 274.—About 7 months old; weight, 230 pounds. Tested with tuberculin prior to inoculation and did not react. On September 15, 1902, 5 c. c. of a suspension of human tubercle culture C No. 4 was injected into right jugular vein. The calf remained well until September 24, 1902, when its respiration was accelerated. Beginning on this date it lost rapidly in condition, became emaciated, lost appetite, and respiration became labored. On October 2, 1902, the animal died and was examined postmortem.

Autopsy: Animal greatly emaciated; the pulmonary pleura was roughened and some portions presented small circular elevations the size of a pinhead. The lung tissue had a granular appearance on section; the dependent portions of the anterior lobes were considerably congested. Liver and kidneys congested. Some of the mediastinal glands congested.

Microscopic examination: Lung—Sections show a great unmber of miliary tubercles, all of which contain large numbers of tubercle bacilli. Liver—Many of the liver cells are undergoing a fatty degeneration; they are granular, and many have lost their nuclei; no distinct tubercles were found, although a number of clumps of tubercle bacilli, as well as scattered separate tubercle bacilli, well stained, were distributed throughout the organ. Spleen—Section shows a number of irregular areas of necrosis which contain large well-stained tubercle bacilli. Mediastinal gland—Some necrotic foci, which contain many large tubercle bacilli. Two guinea pigs inoculated, respectively, with bits of lung and spleen died of generalized tuberculosis.

From the foregoing record it is apparent that calf No. 274 died of a generalized tuberculosis which was brought about by the intravenous injection of human culture C No. 4. The result in this case may also be instructively compared with the results obtained in calves Nos. 260 and 264.

Record of calf No. 260.—Age, 5 months. Tested with tuberculin without reaction. July 19, 1902, 20 c. c. of a suspension in beef broth of bovine culture III was injected into the jugular vein. On July 30 the animal was spiritless, the appetite poor, and respiration accelerated. Beginning on this date the calf lost rapidly in condition, the respiratory disturbance became more marked, and the appetite was lost completely. The animal was found dead on the morning of August 6, 1902.

Autopsy: Postmortem decomposition had progressed so far that it was difficult to make a satisfactory examination. Lungs almost completely solidified; on section they had a granular appearance. Liver had undergone considerable postmortem changes, but did not appear to be diseased. Spleen, kidneys, and lymph glands, with the exception of the mediastinal, appeared to be free from disease.

Microscopic examination: Lung—Smear preparations from this organ showed very many tubercle bacilli. The mediastinal gland also exhibited in smear preparations a considerable number of tubercle bacilli. Sections from the kindey showed neither tubercle formation nor tubercle bacilli, but considerable parenchymatous nephritis was present.

TABLE IV.—*Intravenous inoculations of calves.*

Material used.	Dose.	Number of animal.	Age of animal.	Date of inoculation.	Result of inoculation.
	c. c.				
Human culture F T	5	Calf No. 270[a]	1 year	Sept. 16, 1902	Recovered.
Human culture F L	5	Calf No. 275[b]	9 mos	do	Killed Nov. 3, 1902.
Human culture S E No. 3	5	Calf No. 272[c]	8 mos	Sept. 15, 1902	Recovered.
Human culture G D	5	Calf No. 271[d]	8 mos	Sept. 16, 1902	In moribund condition Nov. 26, 1902; killed.
Human culture 12124	5	Calf No. 289[e]	6 mos	Oct. 14, 1902	Killed Feb. 12, 1903.
Human culture C No. 1	5	Calf No. 273[f]	8 mos	Sept. 15, 1902	Recovered.
Human culture E T	5½	Calf No. 264[g]	5 mos	Aug. 9, 1902	Died Aug. 29, 1902.
Human culture D T	5½	Calf No. 265[h]	5 mos	do	Died Sept. 8, 1902.
Human culture C No. 4	5	Calf No. 274[i]	7 mos	Sept. 15, 1902	Died Oct. 2, 1902.
Bovine culture III	20	Calf No. 260[k]	5 mos	July 19, 1902	Died Aug. 6, 1902.

The following footnotes show the condition of the calves after death:

[a] After recovery from inoculation with human virus received bovine culture subcutaneously. No lesions at autopsy.

[b] Lesions found at autopsy were apparently not progressive.

[c] After recovery received subcutaneous inoculation of bovine culture. At autopsy lesions found in lung, but not severe.

[d] Lesions of tuberculosis not sufficient to account for condition at time animal was killed.

[e] Necrotic areas in lungs. Tuberculous lesions not sufficient to account for poor condition when killed.

[f] After recovery received subcutaneous inoculation of bovine culture. When killed later, found tuberculous lesions; probably due to bovine culture.

[g] Postmortem showed a severe generalized tuberculosis.

[h] Lesions of generalized tuberculosis. The microscope disclosed a contaminating organism, and fatal result may have been partly due to that cause.

[i] The postmortem examination disclosed a generalized tuberculosis.

[k] Tuberculosis involving lungs and some lymph glands.

SUBCUTANEOUS INJECTIONS.

Record of calf No. 250.—About one year old; tested with tuberculin without reaction. April 12, 1902, a piece of tuberculous human lung tissue having a volume of one-half cubic inch was introduced beneath the skin, back of left shoulder of this calf; a piece of thickened tuberculous intestine having a volume of about one-half cubic inch was introduced in like manner back of right shoulder. The diseased lung and intestine were derived from the same case of tuberculosis from which culture C No. 4 was obtained. On May 14, 1902, there was no lesion on the right side at seat of inoculation, but on the left there was a tumor about 3 by 3 inches in area. It had a soft center and had been discharging. The general condition of the animal was poor. As the animal did not improve, it was tested with tuberculin on June 19, 1902, and gave a good reaction. On September 10, 1902, it again gave a partial reaction to tuberculin. On October 1, 1902, the calf was killed and examined postmortem.

Autopsy: At seat of inoculation on left side there was a group of small abscesses in the skin; at seat of inoculation on right side a small subcutaneous tumor with walls of connective tissue streaked with hemorrhagic lines. The contents of the tumor consisted of broken-down necrotic material. The superficial glands in front and back of shoulders and in the flank contained numerous necrotic foci; on the pleuræ of the lung and chest wall were numerous pale-red excrescences, particularly plentiful on the right side, in the dorsal region, and on the posterior border of the principal lobe. Similar growths were attached to the diaphragm; these growths extended about one-fourth to one-third of an inch above the surface of the diaphragm and appeared as isolated, almost circular, elevations about one-half inch in diameter, or as patches made up of numerous similar growths which had coalesced and which varied from one to four inches in diameter. No lesions were found in the lung tissue or in the mediastinal glands.

Microscopic examination: Flank gland—Caseous material from this gland, in which was a considerable amount of lime salts, contained large numbers of tubercle bacilli. Shoulder gland—This gland, which had a caseous center containing a considerable amount of lime salts, also exhibited many tubercle bacilli in cover-glass preparations. Pulmonary excrescences—The section showed a large amount of newly formed connective tissue, many lymphoid cells, and many small blood vessels; there was no evidence of necrotic change; a not inconsiderable number of tubercle bacilli were found.

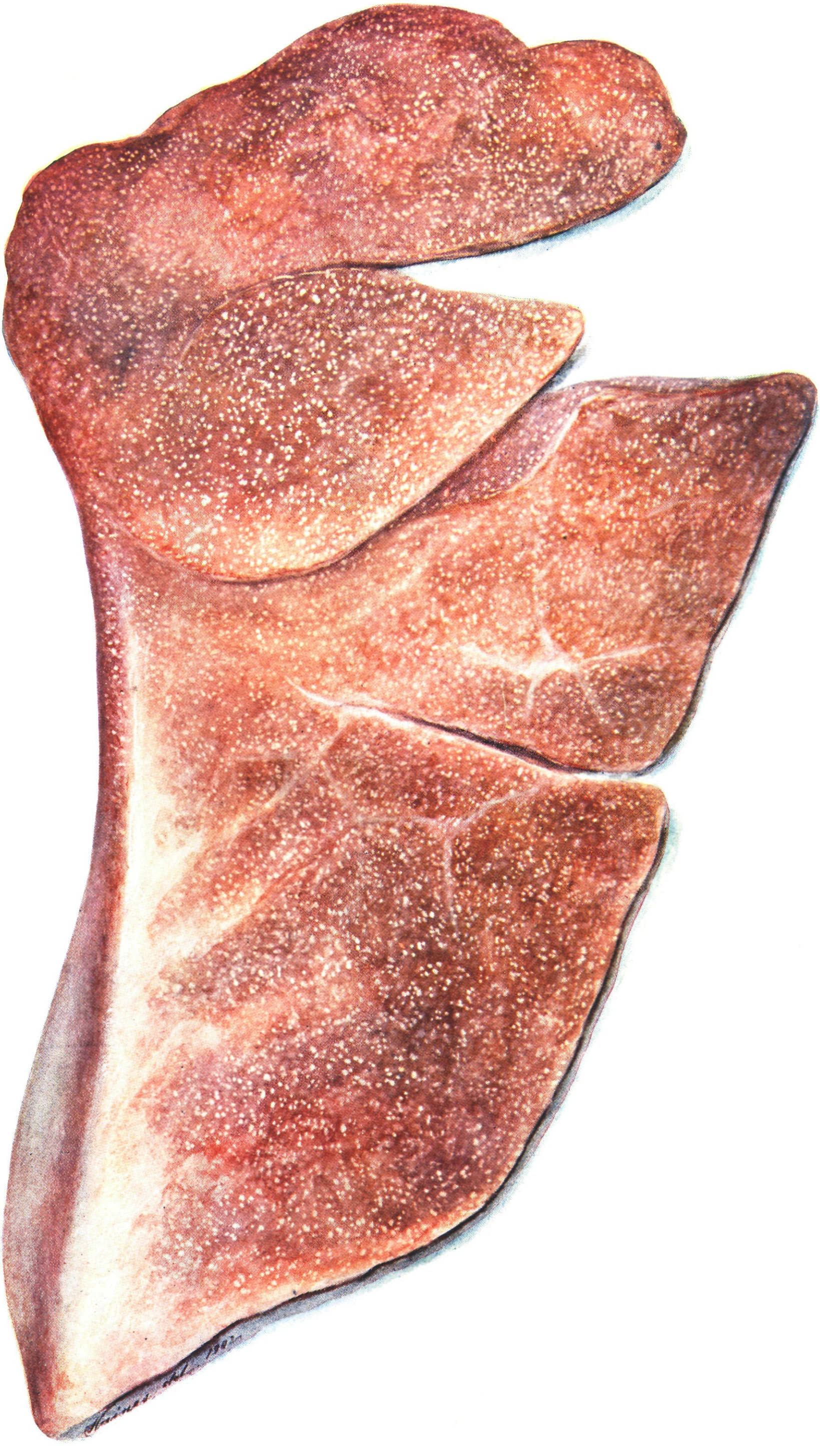
DESCRIPTION OF PLATE XVI.

Sections showing distribution of tubercle bacilli in organs of calf 286, inoculated subcutaneously with C No. 4, human culture. Stained with hematoxylin and carbol-fuchsin. Magnified 1,450 diameters.

Fig. 1.—Section of lung.

Fig. 2.—Section of spleen.

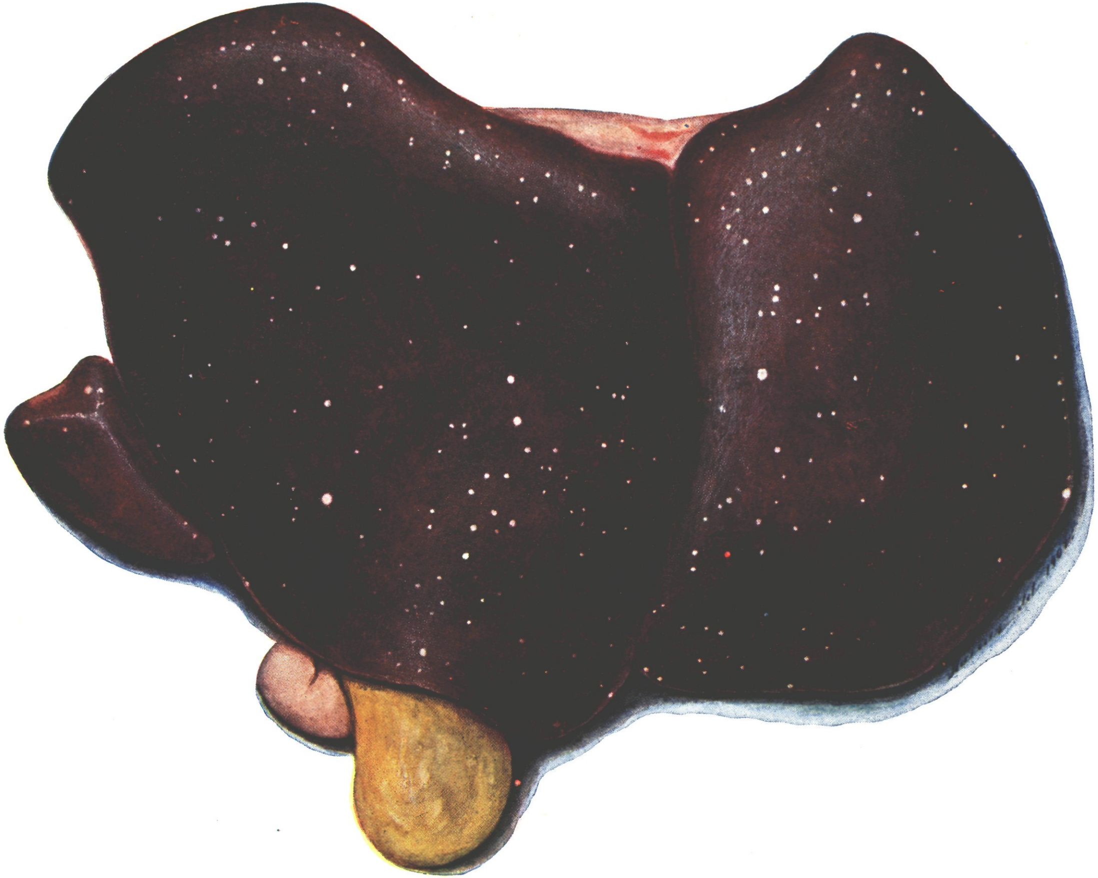
Drawing by W. S. D. Haines.



Lung Calf 286. Subcutaneous inoculation with pure culture human tuberculosis No. C 4.

Bulletin No. 52, B. A. I.

Plate XV.



Liver calf 286. Subcutaneous inoculation with pure culture human tuberculosis. No. C 4.

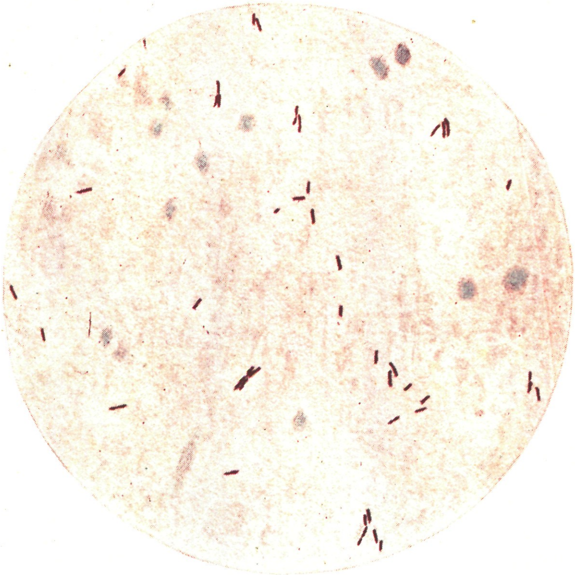


Fig. 1.

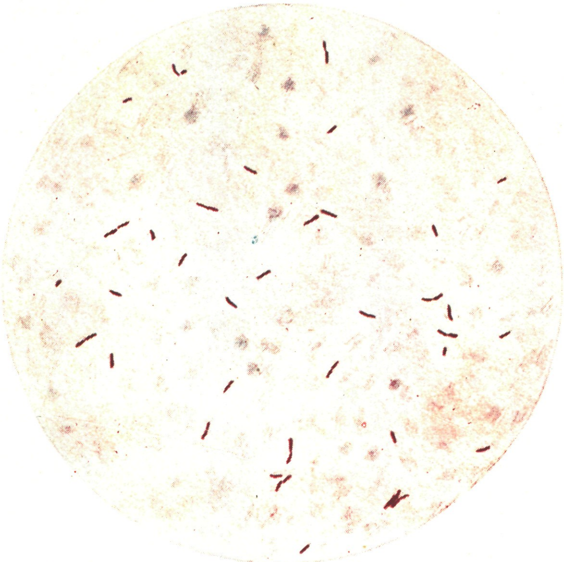


Fig. 2.

Tubercle bacilli in sections from Calf 286. Human tuberculosis.

Diaphragmatic excrescence—The appearance was very similar to that of the pulmonary excrescences, except that the process was not so far advanced, there being a less amount of fibrous tissue and more of the cellular elements; a few giant cells and a very few tubercle bacilli could be seen in different parts of the sections. Guinea pigs inoculated, respectively, with bits of the flank gland, pulmonary excrescence, and diaphragmatic excrescence all died of generalized tuberculosis.

By this inoculation we have produced a tuberculosis which though not fatal, was distinctly progressive in character and we therefore consider this case as one in which we have successfully infected a calf with human tuberculosis. Comparison between the record of this calf and the records of calves Nos. 286 and 274 shows the difference between the result of inoculation with tissues on the one hand and pure cultures on the other.

Record of calf No. 286.—About 6 months old. Tested with tuberculin before inoculation; did not react. On October 13, 1902, this animal was injected subcutaneously on the right side of neck, directly in front of shoulder, with 5 c. c. of a suspension of human tubercle culture C No. 4. On October 23, 1902, this calf had a swelling about 3 by 3 inches in area at seat of injection, and the adjacent prescapular gland was much enlarged. On October 27, 1902, the swelling persisted and the animal was evidently losing in condition. Appetite became very poor and the weakness gradually more pronounced. There seemed to be no disturbance of respiration during the early part of its illness, but on November 14, 1902, the breathing was labored. On this date the calf was unable to stand alone and in a dying condition, and was killed and examined postmortem.

Autopsy: The animal was extremely emaciated; at seat of injection the skin was indurated over an area about 4 inches square; under this induration the gland in front of shoulder was 4 by 3 inches in size. Axillary gland on side of injection was normal; flank gland on same side was normal. Lungs—The entire lungs with the exception of the dorsal portions of the principal lobes were intensely reddened and sprinkled throughout with minute tubercles. Liver normal in color, and sprinkled with numerous small necrotic areas, apparently tuberculous in character, varying in size from mere points to one-eighth inch in diameter. Spleen enlarged and filled with small, round, firm, white nodules, the size of squirrel shot, which are easily picked out of organ. Sprinkled over the omentum were a number of small pearl-gray nodules with reddish centers, probably tubercles. Glandular system—Prescapular gland (immediately beneath point of injection) greatly enlarged; nodular but not caseous; entire gland quite firm. Pharyngeal glands considerably enlarged; a number of small, firm, yellowish nodules in the body of the gland. Mediastinal glands considerably enlarged; the surface was rough; there were no caseous areas. Pubic glands about normal in size, but contained one or two small areas that looked suspiciously like tuberculous foci. Mesenteric glands apparently slightly enlarged. Shoulder gland normal in size and appearance. Flank gland normal. (See Pls. XIII, XIV, XV, and XVI.)

Microscopic examination: Lung—A number of well-defined miliary tubercles were visible in the sections. The central portion of most of the tubercles had undergone a coagulation necrosis and they were in some instances homogeneous and structureless and in others consisted of a mass of nuclear fragments which stained with hematoxylin; all of the tubercles contained a considerable number of rather large tubercle bacilli. Liver—Several large tubercles were found in the sections; the central portions of them were necrotic and they contained a number of well-stained tubercle bacilli. Spleen—This organ contained a number of rather large, well-defined tubercles, most of which had necrotic centers and contained a number of tubercle bacilli, well stained, rather long, many of them beaded. Kidney—A smear from one of the tubercles showed quite numerous tubercle bacilli. Prescapular gland—Large areas of coagulation necrosis, not sharply defined; many well-stained tubercle bacilli were scattered throughout the tissue, more numerous in some places than others. Gland just inside thorax—Some large areas of necrosis and many well-stained tubercle bacilli were found. Pharyngeal gland—A few large, poorly defined areas which were undergoing degenerative changes; a considerable number of well-stained tubercle bacilli could be seen in the affected portions of the tissue. Shoulder gland—No tuberculosis. Flank gland—No tuberculosis. Mediastinal gland—Many tubercle bacilli found. Intestines—No tuberculosis. Suprarenal body—No tuberculosis. Pubic gland—Tubercle bacilli fairly numerous.

From the record of this experiment it will be seen that we have in this instance a generalized tuberculosis in a calf produced by subcutaneous injection of a culture of human tuberculosis. This record should be carefully compared with that of calf No. 284, which was injected subcutaneously with a culture of bovine origin.

Record of heifer calf No. 288.—October 2, 1902; a small animal in fairly good condition, about 6 months old. October 11, 1902, tested with tuberculin without reaction. Injected October 13 subcutaneously on the right side of neck directly in front of shoulder with 5 c. c. of a suspension of human tubercle culture E T. This animal developed an extensive swelling 4 by 5 inches in diameter at the seat of injection. There was, however, very little evidence of general illness. On December 19, 1902, it was killed and examined postmortem.

Autopsy: A tumor 5 by 3 inches at seat of injection; the central portion of this tumor was entirely broken down and filled with a thin, partly cloudy, serous fluid; the walls of the cavity were quite thick and the inner portion was necrotic. Prescapular gland—About normal in size; upon section several yellowish nodules were found. Mediastinal glands—Enlarged; upon section areas of necrosis which contained a considerable amount of lime salts were seen in cortical portion. Lungs—A large number of round, grayish nodules varying from 3 to 8 mm. in diameter were sprinkled over all the lobes of both lungs; these nodules were hard and shot-like, and projected slightly above the surface of the lung; upon section similar nodules were seen to be scattered throughout the organ; the lung tissue, aside from these nodules, showed no lesions. Liver—The color of the main body of the organ was normal; there were, however, scattered over its entire surface a number of round, sharply defined, slightly elevated white areas which varied from 3 to 8 mm. in diameter; upon section similar areas were seen throughout the organ; these nodules were hard and could be distinctly felt projecting above the surface of the liver. Spleen—Normal in size and general appearance; upon section a few firm, round, whitish areas were found, closely resembling the bodies found in spleen of calf No. 286. Kidney—Normal. Intestines—Normal. Mesenteric glands—Normal in size, but upon section a few yellowish areas containing lime salts were found. (See Pls. XVII, XVIII, and XIX.)

Microscopic examination: Lung—A number of large tubercles usually having a distinct surrounding wall of connective tissue, numerous giant cells and necrosis of the center of the tubercles were seen; a very few tubercle bacilli were found. Liver—Tissue generally is in a fair condition; found two rather large areas of circumscribed coagulation necrosis, one of which contained a giant cell; no tubercle bacilli in sections; a few were found in smear preparations from this organ. Spleen—Several tubercles in one section and in one a giant cell; no tubercle bacilli in the section, but a very few in a smear preparation. Kidney—Extensive parenchymatous nephritis with some increase of connective tissue; no tubercles and no tubercle bacilli found. Mesenteric gland—Several large foci of necrosis containing many large giant cells and a very few tubercle bacilli. Point of inoculation—A few tubercle bacilli were noted in smear preparations. Prescapular gland—A few well-stained tubercle bacilli were found in smear preparations.

It is evident that the subcutaneous inoculation of calf No. 288 with human tubercle bacilli brought about a generalized tuberculosis in the animal.

Record of calf No. 285.—October, 1902, red-and-brown heifer about 13 months old, tested with tuberculin without reaction. January 24, 1903, injected subcutaneously in front of right shoulder with 5 c. c. of a suspension of human tubercle culture E T. February 3, 3, 1903, had a lesion at seat of injection which was continuous with the prescapular gland. The latter was about 2 by 5 by 3 inches in size. The general condition of the animal was fairly good. February 13, 1903, prescapular gland and tumor at seat of injection had coalesced, forming a hard enlargement about 4 by 9 by 3 inches in size; general condition poor. February 26, 1903, tumor at seat of injection and nearest prescapular gland have a combined volume of about 6 by 10 by 3½ inches; this enlargement is very hard; general condition is poor; respiration seems to be normal. April 28, 1903, killed and examined postmortem.

Autopsy: Animal in fairly good condition, not fat; at seat of injection in front of right shoulder the lymph gland was about 6 by 5 by 4 inches in size and very firm; a number of small calcareous nodules in the mediastinal glands; the lungs and spleen were dotted here and there with small tubercles.

This animal was inoculated as a control on calf No. 288, and, as will be seen from the record, developed a generalized tuberculosis, which was not so severe, however, as that produced in calf No. 288. Calf No. 285 was about 7 months older than calf No. 288.

Record of calf No. 287.—About 6 months old. October 11, 1902, tested with tuberculin without reaction. October 13, 1902, injected subcutaneously on right side of neck directly in front of shoulder with 5 c. c. of a suspension of human tubercle culture D T. On October 23, 1902, this animal presented a large swelling at the point of injection, and the prescapular gland which was directly beneath this point was enlarged. There were practically no symptoms other than the local lesions as a result of the injection. On December 19, 1902, this calf was killed and examined postmortem.

Autopsy: A large tumor 5 by 3 inches at seat of injection; this tumor was hard, the central portion was necrotic but not softened. Prescapular gland about 3 by 1½ inches; the cortical portion contained tuberculous foci with considerable deposits of lime salts. Gland just inside thorax; about the size of a walnut; a large portion

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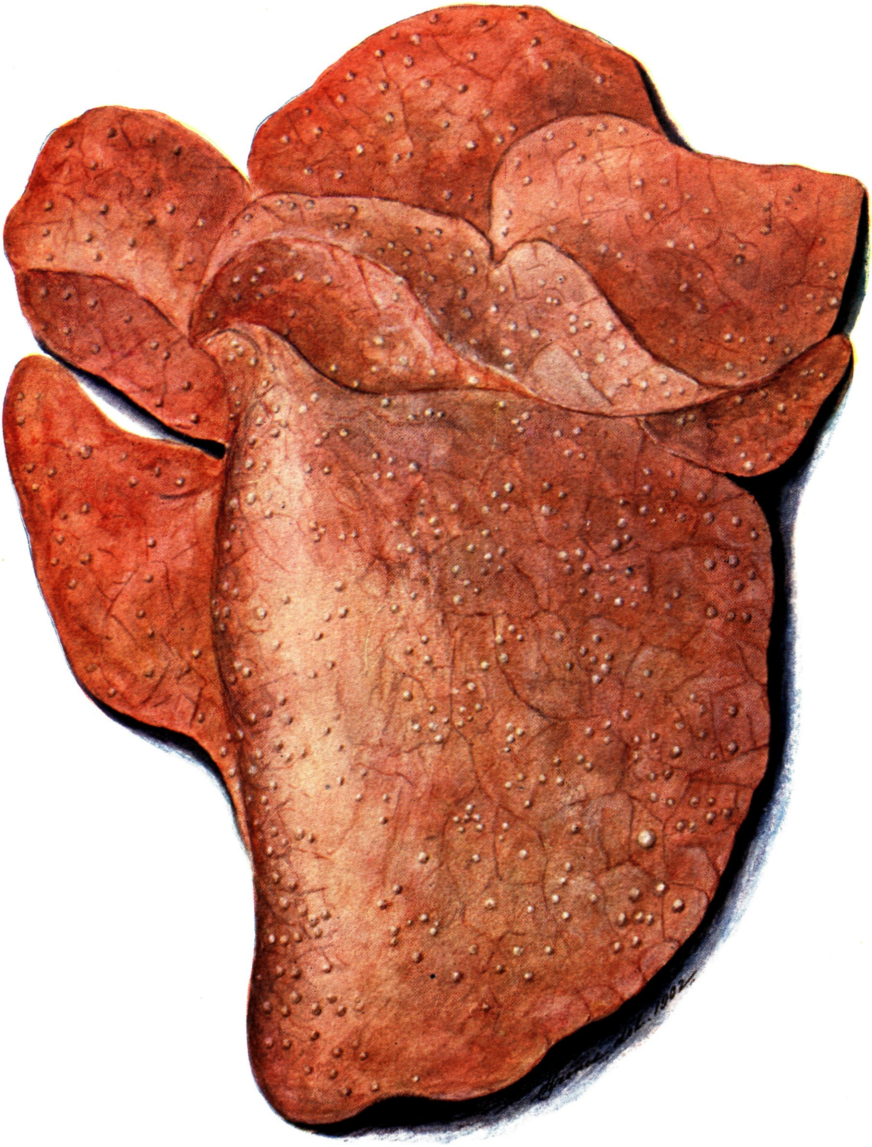
DESCRIPTION OF PLATE XIX.

Reproduction of a smear and a section from tissues of calf No. 288 inoculated subcutaneously with human culture E T, indicating the distribution of bacilli. Stained with hematoxylin and carbol-fuchsin. Magnified 1,450 diameters.

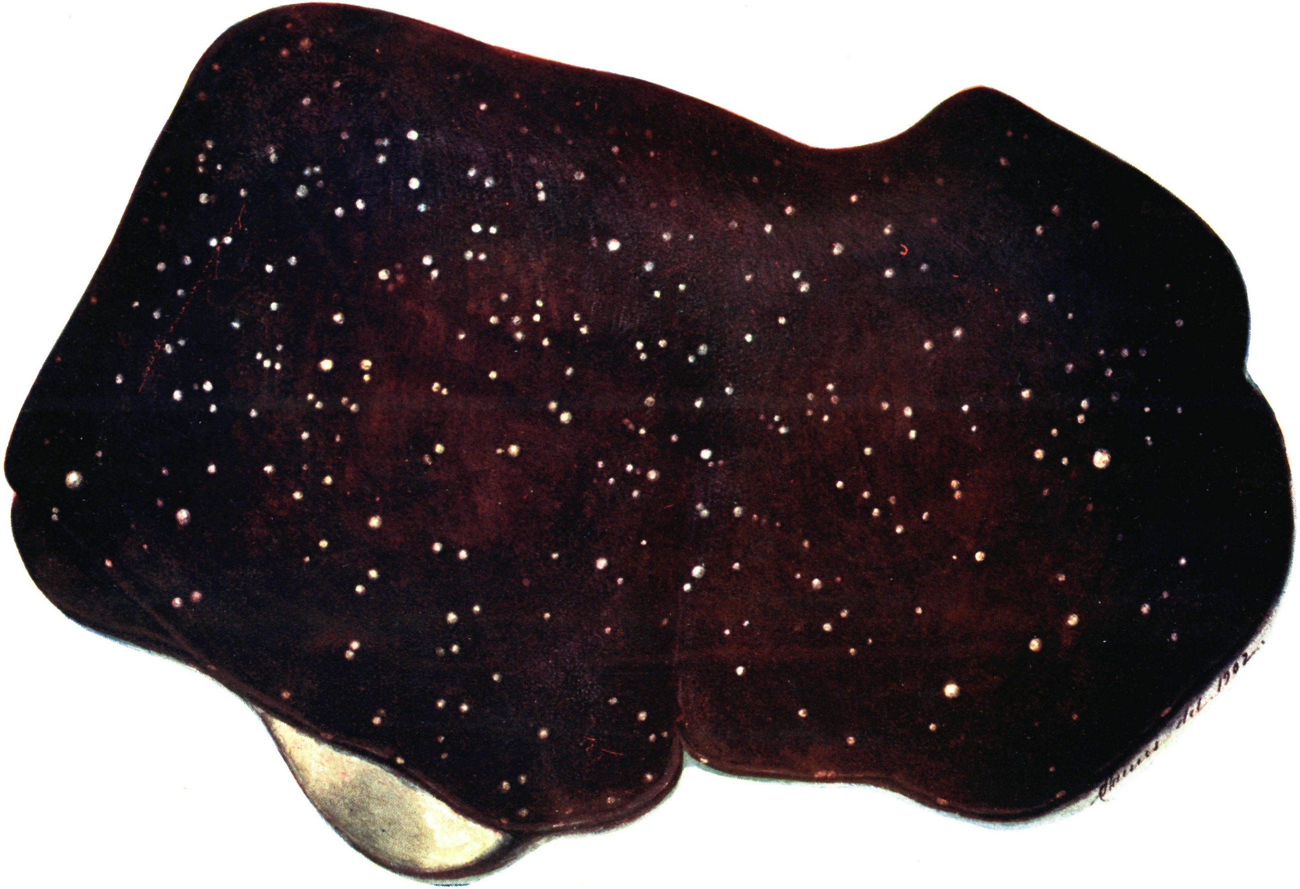
Fig. 1.—Smear made from lung.

Fig. 2.—Section from a mesenteric gland.

Drawing by W. S. D. Haines.



Lung Calf 288. Subcutaneous inoculation with pure culture human tuberculosis No. E. T.



Liver, Calf 288. Subcutaneous inoculation with pure culture human tuberculosis No. E. T.

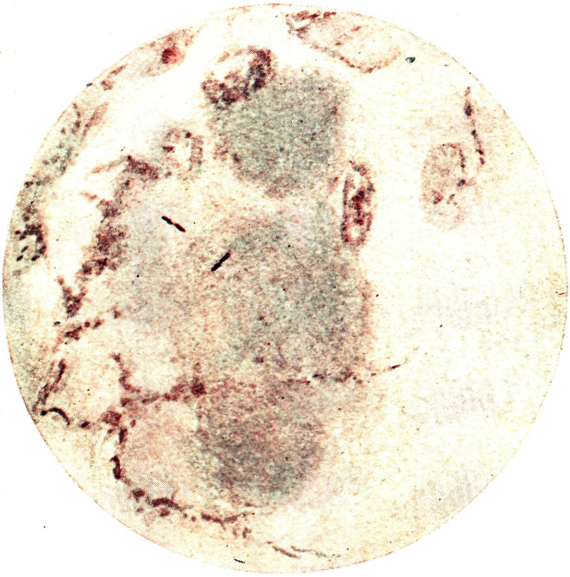


Fig. 1.

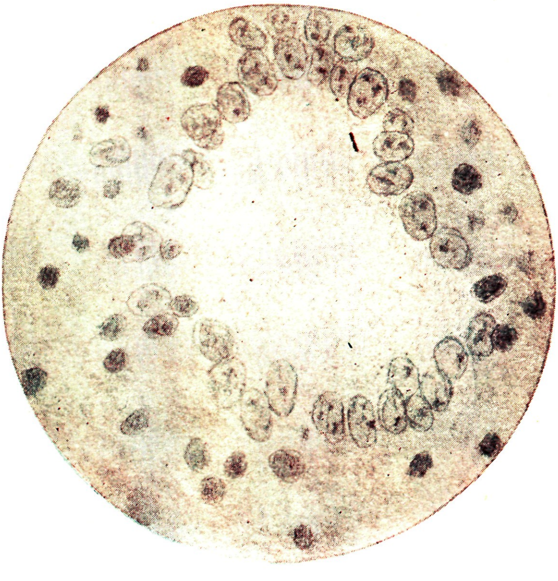


Fig. 2.

Tubercle bacilli from Calf 288. Human tuberculosis No. E. T.

of the gland was necrotic and contained abundant lime salts. This animal was otherwise normal.

Microscopic examination: At point of injection numerous rather large, well-stained tubercle bacilli were found. Smear preparations from prescapular gland contained a few well-stained tubercle bacilli. Gland just inside thorax contained a very few tubercle bacilli.

It is evident that culture D T is distinctly lower in pathogenic power for cattle than either culture E T or C No. 4. It appeared, however, to be possessed of nearly if not quite as great pathogenic power as the rather feeble bovine III culture, and after subcutaneous injection succeeded in invading the lymph glands adjacent to the seat of injection.

Record of calf No. 221.—About 18 months old; gave negative result to tuberculin test. Injected subcutaneously in front of right shoulder with 6½ c. c. of a dilute suspension of human culture G D September 23, 1902. The calf showed no signs of illness as a result of the inoculation. Was tested with tuberculin in June, 1903, and gave no reaction. In November, 1903, the animal being in excellent physical condition, was exposed to natural infection with bovine tuberculosis by being placed in the stall with tuberculous cattle. No symptoms of illness resulted, although several other animals placed under similar conditions contracted tuberculosis.

The calf was killed August 17, 1904, and examined postmortem. No lesions of any kind were found. This experiment was therefore entirely negative in so far as the injection of the calf with human tuberculosis is concerned. The calf evidently possessed considerable resisting power to natural infection. The probability of this immunity having been conferred by the injection of culture G D will be discussed in Part III of this bulletin.

Record of calf No. 290.—About 6 months old; tested with tuberculin without reaction. Injected subcutaneously in front of right shoulder on October 14, 1902, with 5 c. c. of a suspension of human culture F T. No marked disturbance of the health of the animal as a result of the inoculation could be observed. A tumor developed at the point of injection and persisted until June, 1903, when the animal was tested with tuberculin and gave a slight reaction. On July 23, 1903, the calf was killed. The only tuberculous lesions which could be found at autopsy were situated in the subcutaneous tissues at the point of injection.

Record of calf No. 291.—About 6 months old; no reaction from tuberculin test. On October 14, 1902, injected subcutaneously in front of right shoulder with 5 c. c. of a suspension made from human tubercle culture F L. The record of this animal is quite similar to that of calf No. 290. A tumor formed at the point of inoculation, but the health of the calf remained good. When tested with tuberculin on June 30, 1903, a slight reaction was obtained. The calf was killed on July 23 and examined postmortem. The only lesions found were situated in the subcutaneous tissues at the point of injection.

Human cultures F T and F L evidently did not possess sufficient pathogenic power to cause the infection of these two calves after subcutaneous injection.

Record of calf No. 284.—Red-and-white heifer calf, about 13 months old. Tested with tuberculin December 13, 1902, without reaction. January 24, 1903, injected subcutaneously in front of right shoulder with 5 c. c. of a suspension of bovine tubercle culture C No. 81. The injection was followed by the usual local swelling and enlargement of the prescapular gland. The animal in the course of two months failed somewhat in general condition, the respiration became slightly accelerated, and the local lesions persisted. On April 10, 1903, the general condition of the calf was poor and the animal was killed and examined postmortem.

Autopsy: At seat of injection on right side of neck there was an abscess about 3 by 4½ by 2 inches in size; below the abscess the prescapular gland was greatly enlarged and very firm. Lungs were sprinkled throughout with tubercles, which were more numerous in the anterior portions; these tubercles presented a minute pearl-gray center surrounded by a zone of pale-pink edematous tissue. Liver contained a few necrotic foci, which were visible on the surface. Mediastinal glands enlarged. Spleen contained tuberculous foci. Intestines contained numerous worm nodules; no mesenteric disease; a few tubercles, beginning pearly disease, on the omentum over paunch, and a few small tubercles were also found on the costal pleura. (See Pls. XXII and XXIII.)

Microscopic examination: Lung—The sections presented tubercles of varying size which contained giant cells and a few tubercle bacilli. Spleen showed extensive tubercle formation with giant cells and scattered tubercle bacilli. The sections from the liver and kidney showed no lesions.

Record of calf No. 223.—Tested with tuberculin without reaction. Age about two and one-half months. On November 2, 1901, this animal was injected subcutaneously with 10 c. c. of a suspension of bovine culture III (Smith). On January 21, 1902, and April 18, 1902, reactions after tuberculin injections were noted. On October 2, when the animal was killed, its general condition was excellent. At the seat of injection there were embedded small abscesses containing a soft, cheesy material. The shoulder glands on the side of infection, both deep and superficial, were enlarged and sprinkled with necrotic foci. The mediastinal glands contained numerous necrotic foci. No other lesions of any kind were present.

This animal showed, therefore, after eleven months, very much less evidence of tuberculosis than was found after six weeks in calves which received subcutaneous injections of material from tubercle cultures isolated from children.

TABLE V.—*Subcutaneous inoculations of calves.*

Material used.	Dose. c. c.	Number of animal.	Age of animal.	Date of inoculation.	Result of inoculation.
Tuberculous human lung and intestine.	1 piece of each.	Calf No. 250[a]	1 year...	Apr. 12, 1902	Killed Oct. 1, 1902.
Human culture C No. 4.	5	Calf No. 286[b]	6 mos...	Oct. 13, 1902	Killed Nov. 14, 1902, when in dying condition.
Human culture E T...	5	Calf No. 288[c]	....do...	.....do.......	Killed Dec. 19, 1902, in fair condition.
Do	5	Calf No. 285[d]	13 mos..	Jan. 24, 1903	Killed Apr. 28, 1903, condition poor.
Human culture D T...	5	Calf No. 287[e]	6 mos...	Oct. 13, 1902	Killed Dec. 19, 1902, condition fair.
Human culture G D...	6½	Calf No. 221[f]	18 mos..	Sept. 23, 1902	Still living, condition excellent.
Human culture F T...	5	Calf No. 290[g]	6 mos...	Oct. 14, 1902	Killed July 23, 1903, after tuberculin reaction.
Human culture F L...	5	Calf No. 291[g]	....do...	.....do.......	Do.
Bovine culture III	10	Calf No. 223[h]	2½ mos..	Nov. 2, 1901	Killed Oct. 2, 1902, condition excellent.
Tissue from monkey No. 1, inoculated with bovine III.	Small piece.	Calf No. 226[i]	1 mo....	.....do.......	Killed Oct. 6, 1902, condition poor.
Bovine culture C No. 81	5	Calf No. 284[j]	13 mos..	Jan. 24, 1903	Killed Apr. 10, 1903, condition poor.

The following footnotes show the condition of the calves after death:

[a] Tuberculosis of superficial lymph glands, and of the pulmonary and diaphragmatic pleuræ.

[b] The autopsy disclosed a severe generalized tuberculosis.

[c] Autopsy revealed lesions of a generalized tuberculosis.

[d] Generalized tuberculosis. Not so extensive as in case of calf No. 288.

[e] Glands adjacent to point of inoculation were tuberculous.

[f] Animal tested with tuberculin June 30, 1903, and did not react.

[g] No lesions except abscess at point of inoculation.

[h] Abscesses at point of inoculation. Shoulder glands on same side and mediastinal glands tuberculous. No other lesions.

[i] Tuberculosis of lungs, spleen, mediastinal, axillary and shoulder glands.

[j] Generalized tuberculosis.

Record of calf No. 226.—About 1 month old. Given tuberculin test without reaction. November 2, 1901, injected subcutaneously back of left shoulder with tuberculous tissue from monkey No. 1, which died as a result of inoculation with bovine III culture. The calf was tested with tuberculin on the following dates, each time giving a good reaction—January 21, February 27, April 18, and June 26, 1902. Killed October 6, 1902, and examined postmortem.

Autopsy: At seat of injection a group of minute necrotic foci surrounded by thick connective tissue walls, the usual injection lesion produced by bovine tubercle germs. There were numerous minute foci of tuberculosis in the mediastinal glands; entire lung sprinkled with small tuberculous foci; the shoulder and axillary glands were tuberculous; numerous nodules of disease in the spleen; other organs and glands not affected. Two guinea pigs inoculated subcutaneously with a diseased fragment from the spleen of this calf died of generalized tuberculosis.

The widespread lesions found in this calf would seem to indicate an increase in the virulence of bovine III culture by passage through the monkey, inasmuch as a pure culture of the same organism failed to produce generalized tuberculosis in calf No. 223. Without more experimental evidence upon this point, however, we can not regard such an increase in virulence as definitely established.

MISCELLANEOUS ANIMAL EXPERIMENTS.

Record of calf No. 254.—Age, about 7 months. February 27, 1902, tested with tuberculin without reaction. March 7, 1902, injected intraabdominally with 5 c. c. of human sputum containing very many tubercle bacilli; also many other bacteria, staphylococci, etc. Within three weeks after injection the animal appeared to be unwell, appetite was poor, and there was considerable tenderness surrounding the point of injection. On May 1, 1902, the animal reacted to a tuberculin test, and declined in condition from that time on, and was killed on September 23, 1902.

Autopsy: On opening the abdominal cavity the left wall was found to be thickly studded with minute white elevations, varying in size from mere points to about one-eighth inch. Among these elevations were a few which had necrotic centers; similar but less numerous lesions on the omentum, the surface of the rumen, the abdominal and thoracic surfaces of the diaphragm, and on the mesentery; the lesions had very much the appearance of the beginning of pearl disease (see Pl. XX). A few necrotic foci were found in the tissue of the liver near the convex surface. The liver and the stomachs were bound to the diaphragm by means of firm bands of white connective tissue, which were studded with white elevations of the kind already described. The surface of the spleen was marked with similar elevations somewhat larger than in other regions. One cheesy nodule, about 1 inch in diameter, in the abdominal cavity near the duodenum; this nodule on section discharged a thick, granular pus. The walls of the abscess were moderately thick. Lung—The entire cephalic, two-thirds of the ventral, and one-fourth of the principal lobes on both sides were solidified, and of a pale-red color; the solidified areas were sprinkled with nodules, varying in size from one-sixteenth to one-half inch, which on section were found to be dilatations of the bronchial tubes filled with thick viscid mucus. The abdominal and thoracic glands were all enlarged and edematous, but showed no other lesions.

Guinea pigs inoculated with fragments of lung and of a nodule on the peritoneum died of generalized tuberculosis. Calf No. 276, injected with material from the peritoneum of calf No. 254, did not contract tuberculosis.

Calf No. 254 evidently contracted tuberculosis as a result of the injection with human sputum. The disease was not virulent in character, but was plainly progressive. The failure of calf No. 276 to contract the disease when injected with tuberculous tissue from calf No. 254 may have been due to the fact that it received the tubercle bacilli free from other organisms, whereas calf No. 254, which was injected with sputum, received a mixture of tubercle bacilli with various other microorganisms. In view of the fact that in calf No. 254 the tubercle bacilli invaded the lung and the costal pleura after intraabdominal injection, we consider this as a successful transference of human tuberculosis to cattle, although the human tubercle bacilli were aided possibly in their struggle with the resisting powers of the calf by other microorganisms.

Record of steer No. 242.—About 20 months old. The animal was drenched with bovine tuberculous material, obtained from cases of spontaneous tuberculosis in cattle, on January 10, 11, 13, and 21, 1902, and on February 17, 18, and 26 of the same year. Tested with tuberculin a positive reaction was obtained. On October 6, about 9 months after the first drenching, this animal was killed. A few small foci of a tuberculous character were found in the mediastinal and in the lymph glands at the entrance of thorax; otherwise there was no evidence of tuberculosis.

Record of heifer No. 237.—About 2½ years old. November 7, 1901, tested with tuberculin without reaction. This animal was then fed or drenched with tuberculosis material at varying intervals during a period of 6 months, as follows: November 22, 1901, fed with bran mixed with some tuberculous nodules from a human lung. December 23, 1901, a suspension was made in water of several tuberculous nodules from a human liver; a pint of this material was introduced into the stomach of the calf. December 26, 1901, drenched with a pint of a watery suspension of human tuberculous material, together with some serous fluid from the same case of human tuberculosis. January 8, 1902, drenched with a suspension of a tuberculous human liver. February 11, 1902, a suspension of tuberculous human lung in about 1 gallon of water, with about 50 c. c. of tuberculous human sputum, from adults suffering from pulmonary tuberculosis was introduced into the animal's stomach through a rubber tube. February 27, 1902, a tuberculin test showed no reaction. On March 4, 1902, the calf was drenched with a watery suspension of a portion of a tuberculous human lung. March 7, 1902, about 100 c. c. of tuberculous human sputum was fed to the calf through a rubber tube. About half of the sputum was derived from the same source as that used for the injection of calf No. 254; the other half was obtained from the case that furnished the material from which culture F L was obtained. Both contained large numbers of tubercle bacilli. April 5, 1902, a tuberculin test failed to give a reaction. On April 12, 1902, the heifer was drenched twice with tuberculous material of human origin; the first time it received a suspension of 8 to 10 cubic inches of a tuberculous human lung in water; at the second drenching, an hour later, it received a suspension of tuberculous intestines and mesenteric glands from a child, the latter material being derived from the same source as was culture C No. 4, the culture proving quite virulent for calves when injected subcutaneously. On May 13, 1902, a considerable amount of human tuberculous material (consisting of portions of the lung, liver, spleen, and kidney from a case of generalized tuberculosis) suspended in water was fed to the animal. May 19, 1902, about 8 or 10 cubic inches of a tuberculous human lung and mesenteric glands were cut into small fragments and mixed with water and fed to the calf. On June 26, 1902, tested with tuberculin without reaction.

The general condition of this animal remained excellent; it was killed on October 28, 1902, and the autopsy revealed no trace of tuberculosis. It must be said with regard to the material used for feeding the animal that none of it was proved to possess noticeable pathogenic virulence for cattle upon subcutaneous injection, with the exception of that marked C No. 4. Previous to receiving the virulent material, however, many feedings of a tuberculous virus of a low degree of virulence had been given, and there is a possibility that a certain amount of resistance to tuberculosis had been acquired in this way; in any case one feeding experiment of this character can not be considered as in any way decisive, the individual resistance of the animal being a quantity which must always be reckoned with.

Record of sheep No. 41.—January 29, 1902, tested with tuberculin without reaction. July 19, 1902, injected 10 c. c. of human tubercle culture No. 50 into right jugular vein. As a result of this injection the animal developed a severe respiratory disturbance and lost considerably in condition. At times it seemed to improve, but failed again and gradually became very emaciated. On January 6, 1903, the sheep was killed and examined postmortem.

Autopsy: Lung—The pleura showed numerous patches of thickening which in many places looked like fibrinous neoplasms of a shreddy character, and which constituted in some cases adhesions between the costal and pulmonary pleuræ; the lung tissue was sprinkled with nodules of a necrotic tuberculous character varying in size from one-sixteenth to one-half inch. (See Pl. XXIV.) Mediastinal gland tuberculous; some white points in the liver, kidney, and spleen; the general appearance was that of a tuberculosis which had its origin in the lung, spreading from this organ and finally producing a generalized miliary tuberculosis.

TABLE VI.—*Miscellaneous animal experiments.*

Animal.	Material used.	Method of administration.	Age of animal.	Date of exposure to infection.	Result.
Calf No. 254[a]	Tuberculous human sputum.	Intraabdominal (5 c. c.).	7 mos	Mar. 7, 1902	Killed Sept. 23, 1902.
Heifer No. 237[b]	Tuberculous human tissue and sputum.	By mouth	2½ years.	Nov. 22, 1901	Killed Oct. 28, 1902.
Steer No. 242[c]	Tuberculous bovine tissue.	do	20 mos	Jan. 10, 1902	Killed Oct. 6, 1902.
Sheep No. 41[d]	Human tubercle culture 50.	Intravenous (10 c. c.).	Old ewe.	July 19, 1902	Killed Jan. 6, 1903.

The following footnotes show the condition of the animals after death:

[a] Tuberculosis of peritoneum, lungs, and pleuræ.

[b] No tuberculosis.

[c] A few small tuberculous foci in mediastinal and anterior thoracic glands. No other lesions.

[d] Generalized tuberculosis.

As will be seen from the foregoing records, several drenching or feeding experiments with cattle were made both with human and bovine tuberculous material. At the time the animals were killed the human material had not caused any disease, while the bovine material had produced slight results. When we compare the disease produced by bovine III culture, which had been grown in the laboratory of the Bureau for a number of generations, with that produced by bovine culture C No. 81—the culture recently isolated—there seems to be almost as great a difference in virulence between the two as there is among the various cultures of human origin.

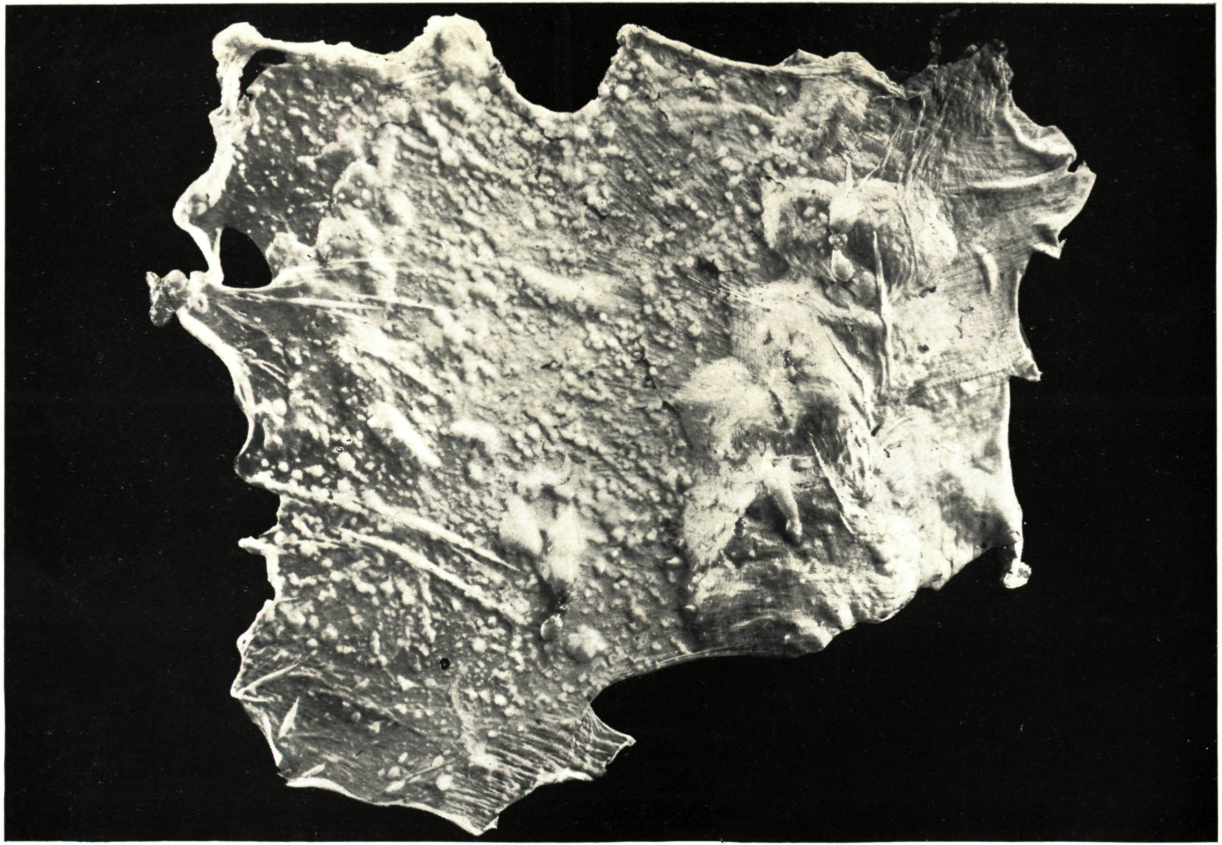
DISCUSSION OF THE CATTLE EXPERIMENTS.

We isolated in all nine fresh tubercle cultures from as many different cases of human tuberculosis. Five of these cultures were obtained from adults and four from children. One calf was injected intravenously with each of the cultures, but owing to the fact that the microscopic examination of the organs of calf No. 265, injected with human culture D T, revealed the presence of large numbers of a microorganism differing from the tubercle bacillus, we will not take this case into account in determining the percentage of positive results. An examination of the record of calf No. 287, injected subcutaneously with the same culture—D T—indicates that this culture possesses distinct though rather feeble pathogenic power for cattle. If the record of calf No. 287 be compared with that of calf No. 223, injected subcutaneously with bovine culture III, it will be seen that, assuming the resisting powers of the two calves to be equal, human culture D T is nearly, if not quite, as virulent for calves upon subcutaneous injection as bovine culture III, and this bovine culture, upon intravenous injection, produced an infection of the lungs and lymphatic glands. Reasoning in this fashion, we are inclined to believe that human culture D T was the chief cause of the lesions found in calf No. 265, but owing to the element of uncertainty, which can not be overcome, this animal will not be considered among the intravenous injections.

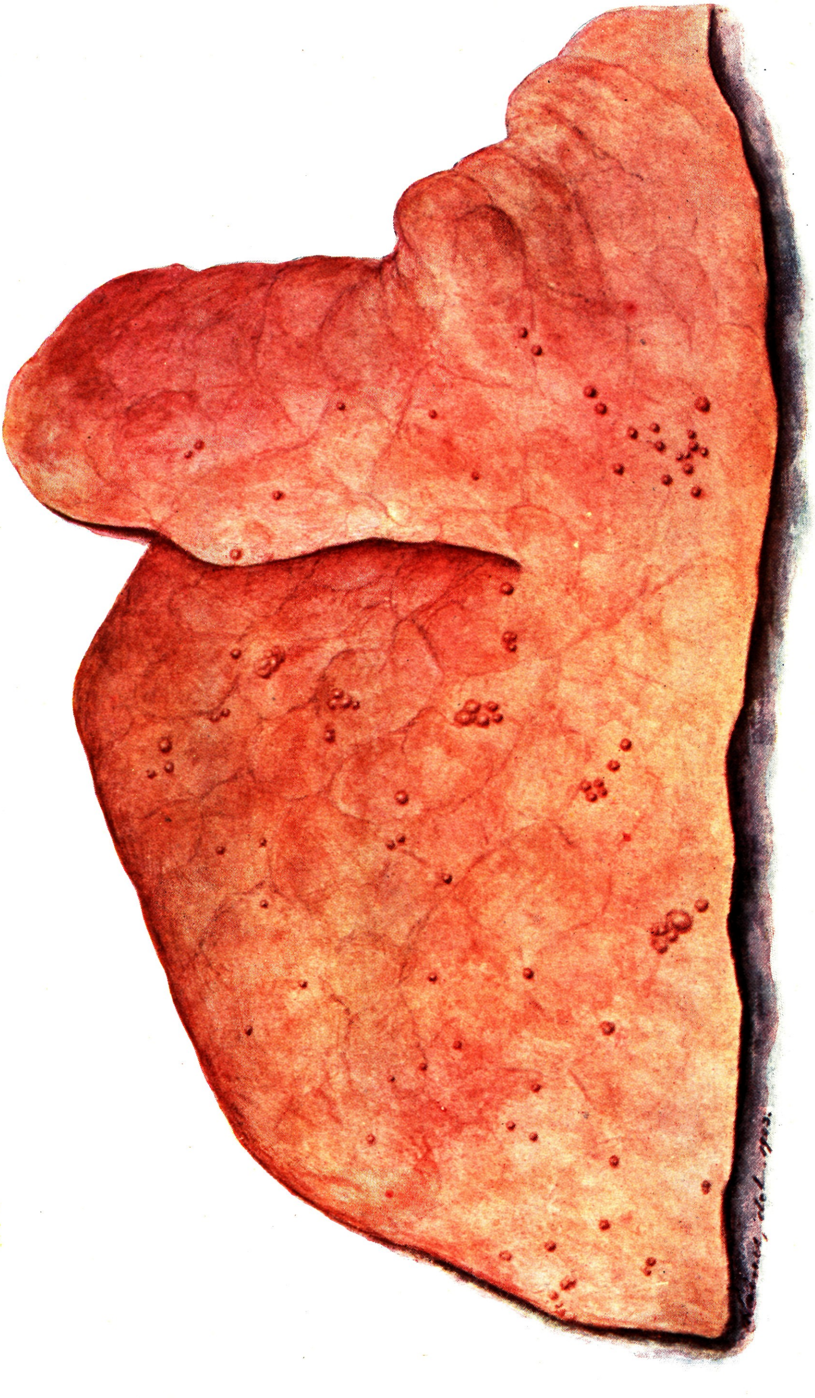
Eight calves were injected intravenously with as many different cultures of tubercle bacilli of human origin, five being obtained from adults and three from children. Of the eight cultures, two, or 25 per cent, produced a fatal generalized tuberculosis. The two that produced fatal disease in calves were both derived from children; therefore two out of three of the cultures from children were quite as virulent for calves as the bovine tubercle bacillus.

It was not thought necessary to inject calves subcutaneously with the cultures which gave little or no evidence of pathogenic properties when injected intravenously. Six of the most virulent human cultures were therefore selected for the subcutaneous injections. As indicated in Table V, seven calves were injected subcutaneously with the six cultures, and one calf (No. 250) was injected with human tuberculous tissue obtained from the same source as one of the cultures (C No. 4). Three of the six cultures used were derived from adults and three from children. The results of these injections may be summarized as follows: One culture failed to produce any lesion persisting for as long a period as 9 months; two produced a localized abscess at the point of injection; a fourth culture produced a local lesion and the disease extended to the neighboring lymph glands. The two remaining cultures brought about a generalized tuberculosis in the calves injected with them. Taking into account, then, the whole number of human cultures isolated, we find that two out of nine, or 22.2 per cent, produced a generalized tuberculosis after subcutaneous injection. A third culture, D T, must be considered as infectious for cattle, as the disease within 2 months from the time of injection extended from the point of inoculation to the adjacent prescapular gland, and through that or some other channel to the deeper gland just inside of the thorax. We can then revise

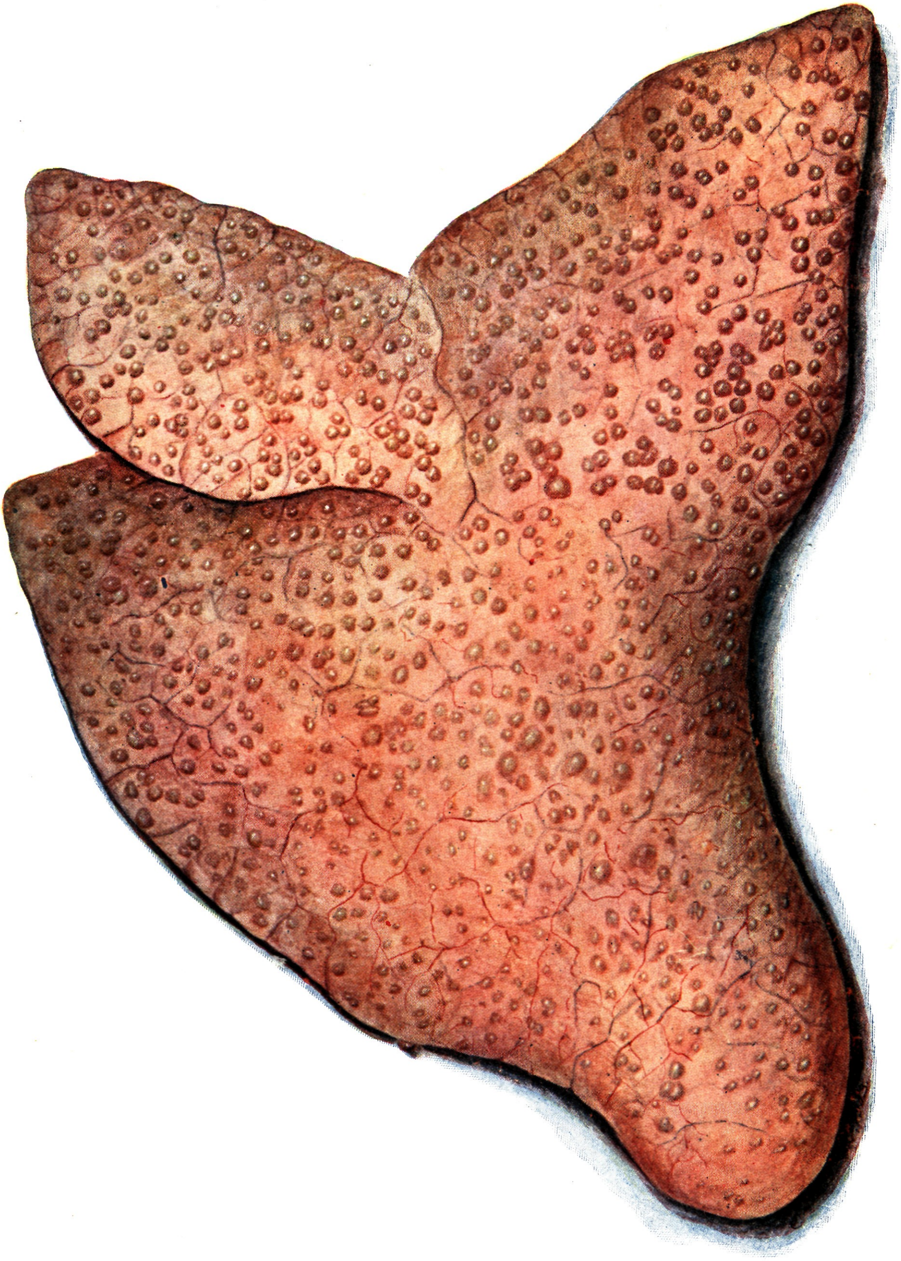
Plate XX.



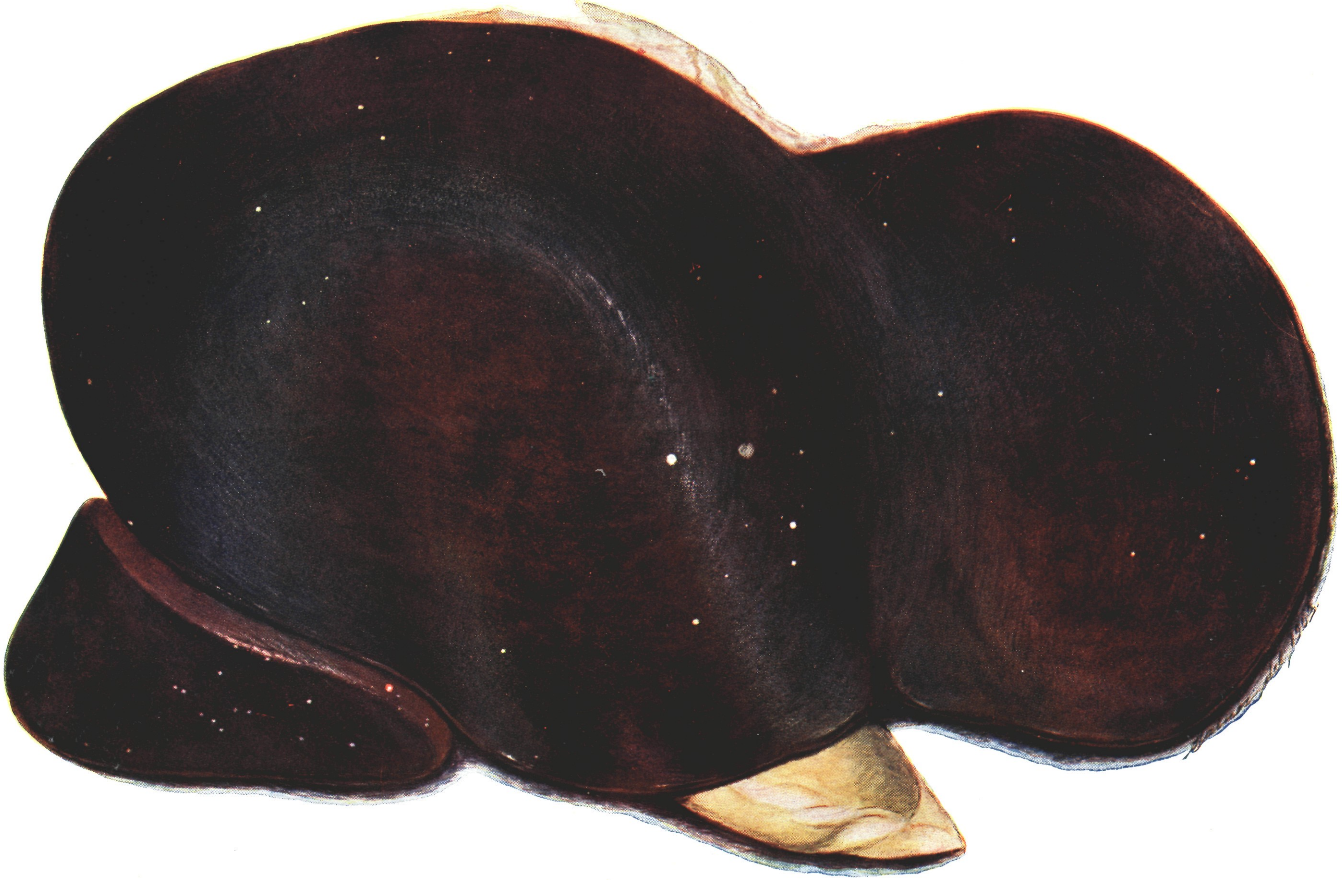
Peritoneum from Calf No. 254, Showing the Large Number of Tubercles Produced by the Intraperitoneal Injection of Human Tuberculous Sputum.



NATURAL BOVINE TUBERCULOSIS IN LUNG OF COW.



Lung, Calf 284. Subcutaneous inoculation with pure culture bovine tuberculosis No. 81.



Liver, calf 284. Subcutaneous inoculation with pure culture bovine tuberculosis No. 81.

the figures just given, since out of nine human cultures isolated, three, or 33⅓ per cent, produced tuberculosis in cattle after subcutaneous injection. These three virulent cultures were all obtained from children, so that out of four cultures derived from cases of generalized tuberculosis in children three were capable of bringing about infection in cattle.

It will be remembered that in the summary of the intravenous injections it was stated that two out of three of the cultures from children produced tuberculosis in calves, while after subcutaneous injection we state that three out of four produced infection. This discrepancy is due to the fact previously explained that a mixed infection appeared to have taken place in the animal injected intravenously with culture D T, and for this reason the injection with that culture was thrown out.

So far as our experiments upon cattle are concerned, therefore, we must conclude that tubercle bacilli of various degrees of virulence for cattle may be obtained from human sources—some quite as virulent as the tubercle bacilli known to be of bovine origin and others less so.

The results of our experiments also lead us to conclude that there is no essential difference between tubercle bacilli derived from bovine sources and those derived from man. In fact, we know of no character possessed by bovine tubercle bacilli which may not be possessed by tubercle bacilli of human origin. The two most virulent human tubercle cultures described in this bulletin can not be distinguished from the classical bovine type either with regard to their cultural characters or their pathogenic power for cattle and other animals.

Dr. Theobald Smith has been the chief advocate for a racial distinction between bovine and human tubercle bacilli, although he does not deny the infectiousness of bovine tubercle bacilli for man. On the contrary, he considers those cultures of human tubercle bacilli which correspond culturally and in respect to their pathogenic powers with the ordinary bovine tubercle bacillus as being originally of bovine origin.

While we believe firmly that tubercle bacilli of bovine origin may infect human beings, we can not accept the theory that tubercle bacilli derived from man are necessarily of bovine origin because they possess characters of the ordinary bovine bacillus. In other words, in regard to those cases of human tuberculosis from which bacilli virulent for cattle are obtained, it must be shown first of all that the individuals in question became infected through tuberculous meat or milk. The possibility of the infection having been derived from cattle always exists, but in our opinion the biological characters of the infecting organism are not sufficient to show that meat or milk was the source of the disease, and in like manner a tubercle bacillus of low virulence obtained from cattle should be considered to be of bovine origin unless there is some evidence of infection from other sources. The fact that certain tubercle bacilli derived from man resemble the majority of bovine bacilli is to our mind evidence of the unity of the two races.

EXPERIMENTS UPON MONKEYS.

In addition to the investigations made to determine the extent to which tubercle bacilli of human origin are infectious for cattle, the intimately related question whether tubercle bacilli of bovine origin are infectious for man was approached as closely as possible by a series of experiments with monkeys.

It is a generally admitted zoological fact that man stands in closer relationship to the quadrumana than to other animals. With this view accepted, we may reasonably assume that apparently similar disease-producing microorganisms found in man and cattle, the identity of which has been questioned, are practically the same in their pathogenic significance if they produce identical affections in monkeys.

Of the monkeys used in our experiment one was a Cebus, two belonged to the genus Rhesus, and four were baboons. Two tubercle cultures were used, one—No. 50—of human origin, which had been under cultivation for a long time at the laboratory and was virulent for guinea pigs, and one bovine culture—III.

Four of the monkeys received injections of bovine tubercle culture; two received injections of human tubercle culture, and one was fed milk containing bovine tubercle bacilli. No cow could be found with a tuberculous udder which had become infected in the natural way at the time the feeding experiment was made, and so the milk was obtained from a cow whose udder was injected with culture bovine III. The milk from the udder-injected cow showed the presence of tubercle bacilli on microscopic examination, and on injection into guinea pigs caused fatal, generalized tuberculosis.

The monkeys were confined in large cages, each of which was supplied with perches and a partly closed sleeping compartment to which the animal could retire at will. The cages were placed, in a one-roomed, properly heated, well-lighted and ventilated house, which was guarded during cold weather against sudden changes of temperature by a small vestibule with inner and outer doors. Both the cages and the house were specially constructed for this experiment and had not been used previously for any other purpose.

Record of monkey No. 1.—Female baboon, in excellent condition, tested with tuberculin without reaction, kept under observation about two weeks and showed no symptoms of disease; weight 8 pounds 9 ounces. September 12, 1901, injected subcutaneously, left side, about 1 inch below udder, with 1 c. c. tubercle culture bovine III.

A firm tumor at once developed at the seat of injection, which gradually increased in size, softened, broke open, and discharged a creamy, gray, pus-like substance, and formed, by October 4, a deep ulcer, 1 by ½ inch in area, with a thickened, rounded, raised border. The skin about the ulcer became generally thickened and detached from the subcutaneous structures over a diameter of 2½ inches. The monkey spent much time fingering and picking the wound, which was washed with weak solution of carbolic acid and covered with a thin layer of powdered chalk and alum. The axillary lymph gland on the side of injection increased greatly in size and was clearly visible as a tumor under the skin when the monkey's arm was drawn upward. On October 8 the ulcer at the seat of injection was almost circular, 1½ inches diameter, and its surface was covered by a thin layer of thick, gray pus. The enlarged axillary gland measured 1 by ¾ by ½ inch, and was firm and nodular to the touch. On October 12 the weight of the monkey was 7 pounds 15 ounces, and on October 26 this had fallen to 7 pounds.

The general condition of the monkey, beginning a few days after the injection until October 30, 1901, on which date she was chloroformed and examined postmortem, became gradually worse. On October 29 the following record was made: Monkey has been growing constantly weaker and now is so weak that she can barely sit with her body in an erect position. She is drowsy, keeps her eyes closed, and rests her head on her hands in a very human attitude, and refuses all nourishment excepting a little fruit. The next day, on which she was killed, she was noticeably weaker and practically in a dying condition.

Autopsy: At the seat of injection a large ulcerated area, about 1½ inches in diameter. The pleural cavity contains a viscid, serofibrinous exudate, by means of which the costal and pulmonary pleuræ are bound together. The lung is thickly sprinkled with opaque, yellowish white areas, of a cheesy appearance, the diameter of which varies from 1 mm. to about 2 cm. The several lobes of the lung are adherent to each other and the lung tissue is firm and airless. Pericardium distended with clear, serosanguinous fluid. Heart—Near the apex of the left ventricle, in the muscle, is an opaque, white area about 2 mm. in diameter. Liver apparently enlarged and mottled with numerous opaque, white areas from 1 to 5 mm. in diameter. Spleen apparently enlarged and sprinkled with opaque, dirty-white areas, which have a cheesy appearance and vary in diameter from 1 mm. to 1 cm. Kidneys pale and studded with opaque, white areas about 1 mm. diameter. The mediastinal, axillary, and mesenteric lymph glands are enormously enlarged, and are hard and cheesy.

The mesentery is sprinkled with opaque, white areas. The brain is normal in appearance, with the exception of a white focus 1 mm. in diameter embedded in the pia mater lying over the subfrontal sulcus.

Record of monkey No. 2.—Small female Cebus, in unthrifty condition, hair rough and soiled in places, tested with tuberculin without reaction; weight 2 pounds 1 ounce. September 12, 1901, injected subcutaneously, left side, about 1 inch below udder, with ½ c. c. of tubercle culture bovine III.

Condition as a result of the injection is similar to that described for monkey No. 1, in addition to which the mammary gland directly above the seat of injection is swollen and firm and the skin covering it, especially about the nipple, reddened. This animal gradually lost in condition, but remained fairly bright until the latter part of October. On October 12 its weight was 1 pound 14 ounces, on October 26 also 1 pound 14 ounces. On November 7 the following record was made: Weight 1 pound 12½ ounces; condition very poor and unthrifty; hair roughened and much soiled; mammary gland greatly swollen and the skin over same, especially about the nipple, intensely reddened; ulcer at the seat of injection 1 inch long and ¾ inch broad; appetite very poor. On November 22 the monkey was in a dying condition and was chloroformed and examined postmortem.

Autopsy: The animal is very much emaciated. At the seat of injection is a large raw, ulcerated area, about 1 inch in diameter, over which the skin has entirely disappeared; the edges of the skin surrounding the opening are somewhat everted. Lung sprinkled with grayish, translucent foci from 1 to 20 mm. in diameter, the centers of which are opaque and of a yellowish white color. Liver apparently normal. Spleen of a dark color and studded with opaque yellowish white areas from 1 to 3 mm. in diameter. Kidneys pale and contain a number of translucent areas about ½ mm. in diameter; the left kidney, on section, is found to contain a yellowish white, opaque, necrotic area about 3 mm. in diameter. Lymphatics—The axillary, mediastinal, and mesenteric glands are all enlarged and cheesy, and all, on section, show more or less softening and the presence of thick, white pus.

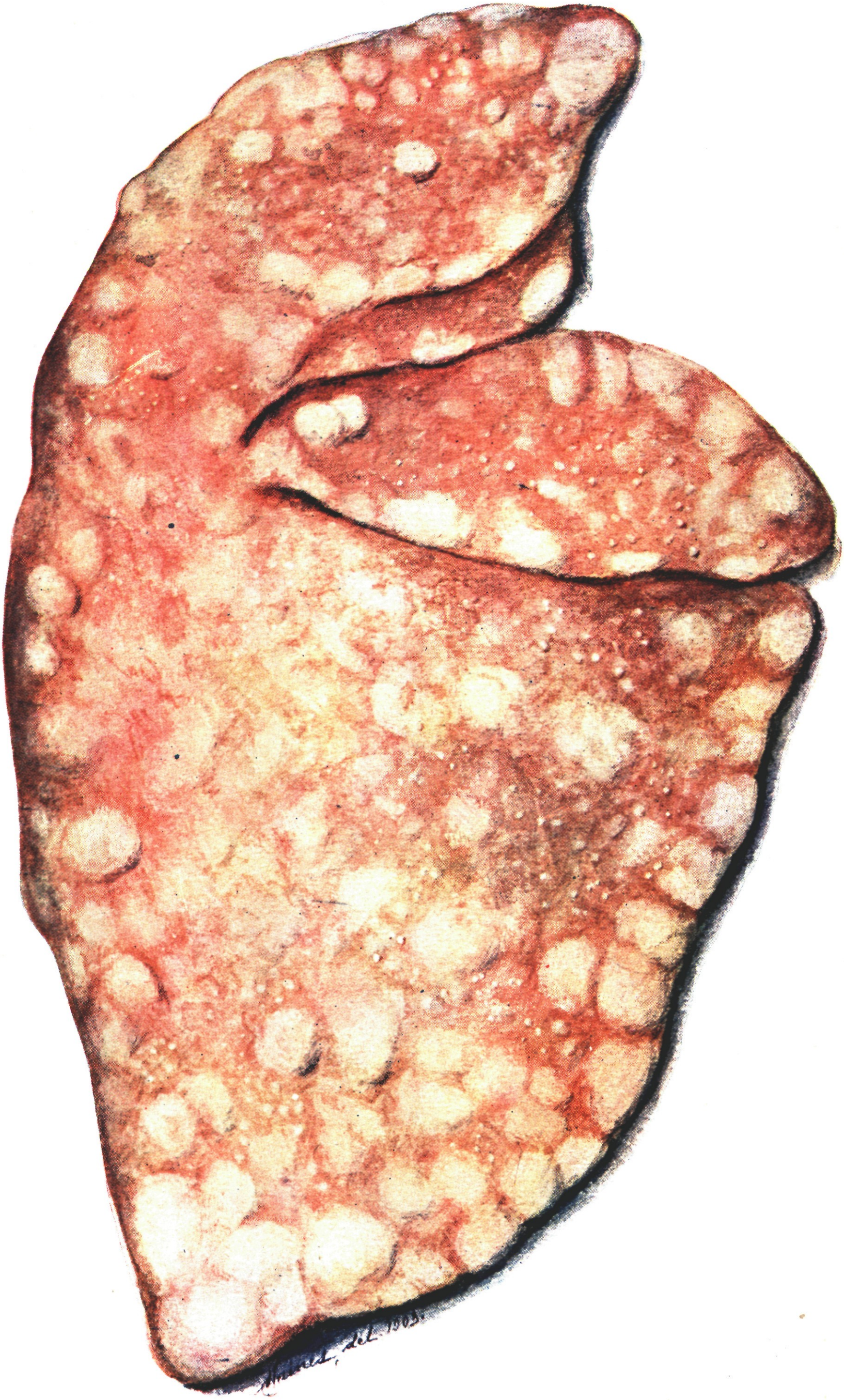
DESCRIPTION OF PLATE XXVI.

Sections of organs of monkey No. 3, indicating distribution of tubercle bacilli from subcutaneous inoculation with pure culture human tuberculosis No. 50. Stained with hematoxylin and carbol-fuchsin. Magnified 1,450 diameters.

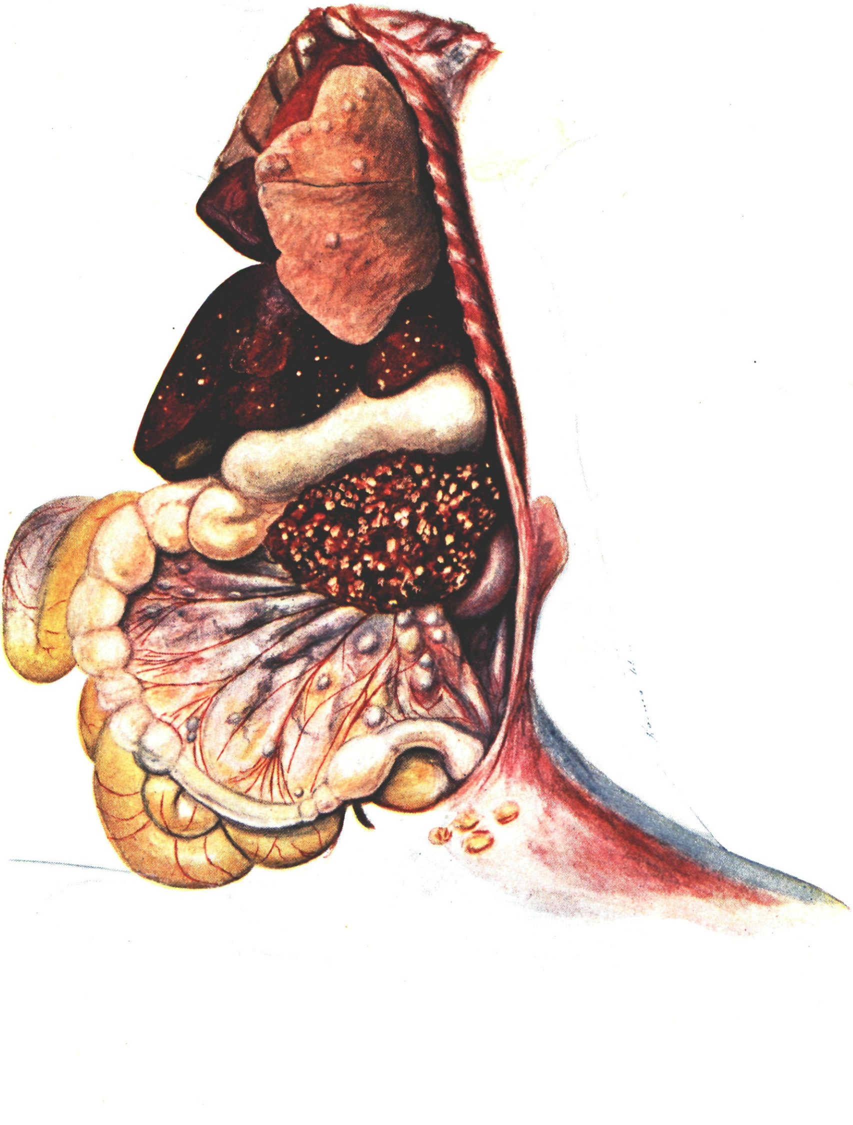
Fig. 1.—Section of lung.

Fig. 2.—Section of liver.

Drawing by W. S. D. Haines.



Lung, Sheep 41. Intravenous inoculation with pure culture human tuberculosis No. 50.



Monkey No. 3. Baboon, subcutaneous inoculation with pure culture human tuberculosis No. 50.

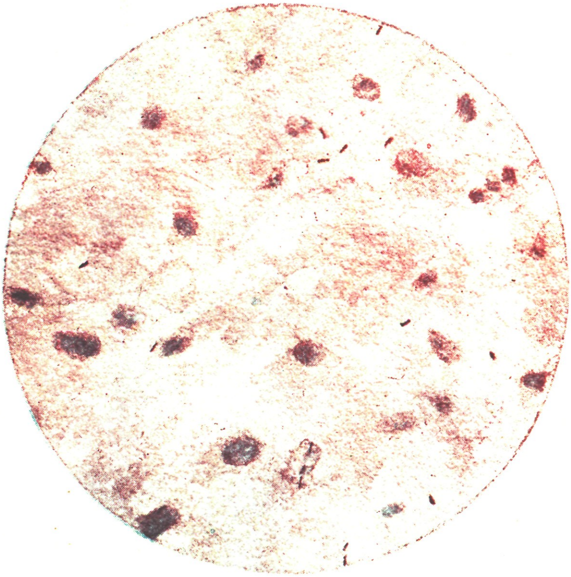


Fig. I.

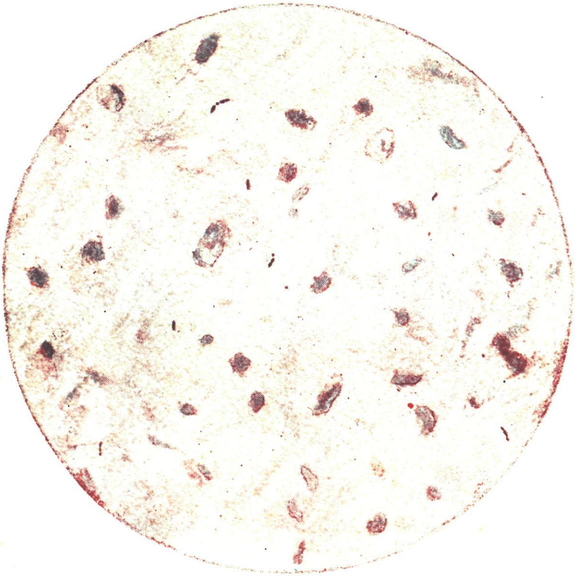


Fig. 2.

Tubercle bacilli in sections from Monkey No. 3, human tuberculosis No. 50.

Record of monkey No. 3.—Female baboon, in excellent condition, tested with tuberculin without reaction; weight 9 pounds 8 ounces. November 7, 1901, injected subcutaneously inside of right thigh with 1 c. c. of human tubercle culture No. 50.

November 20, at the seat of injection an ulcer, ½ by ¾ inch, has formed, in all respects similar to the ulcer described at the seats of injection of monkeys Nos. 1 and 2. The superficial inguinal gland near the seat of injection is greatly enlarged and very firm. From this time on the ulcer and inguinal gland gradually increased in size and the inguinal gland on opposite side showed some swelling. On December 4 the weight had fallen to 7 pounds 14 ounces, and the animal was depressed and had a severe cough which lasted until death. On December 18 the weight was 7 pounds 7 ounces. On December 28 the monkey was very drowsy, depressed, and weak, and had lost her appetite entirely, and was suffering with a diarrhea, the discharges of which were of a creamy consistency and a pale-yellow color. On December 30, being in practically a dying condition, she was chloroformed and examined postmortem.

Autopsy: General condition very poor; weight 6 pounds 15 ounces. An ulcer at seat of injection of such depth that the muscles of the thigh are exposed over an area 3 cm. in diameter; an abscess in the inguinal region, near the ulcer, about 2 cm. in diameter. Inguinal gland near seat of injection enlarged and tuberculous. Lung sprinkled with tubercles varying greatly in size. Spleen filled with caseous tubercles. Liver sprinkled with small white tubercles. Kidneys contained a few small tubercles. Mesenteric, mediastinal, and bronchial glands are enlarged and caseous. (See Pls. XXV and XXVI.)

Record of monkey No. 4.—Male baboon, in excellent condition, tested with tuberculin without reaction; weight 9 pounds 12 ounces. November 7, 1901, injected subcutaneously inside of right thigh with 1 c. c. bovine tubercle culture III (14).

November 20, an abscess, 15 mm. in diameter, has formed at seat of injection, from a small opening in which a thin, brown fluid is being discharged. The superficial inguinal gland near seat of injection is firm and appears as a tumor, about 1 cm. in diameter, under the skin. Weight of animal this date is 9 pounds 15½ ounces. December 4, weight 9 pounds 9 ounces. The lesion at seat of injection is now an ulcer, in all respects similar to the injection ulcers described in connection with monkeys Nos. 1, 2, and 3. The inguinal gland near seat of injection is greatly enlarged, the animal is dull, and suffers with paroxysms of coughing which last several minutes at a time. December 18, weight 9 pounds; monkey becoming weaker and showing practically the same symptoms observed during the last week of life in monkey No. 3—depression, weakness, drowsiness, cough, and diarrhea. December 23, in practically dying condition, chloroformed and examined postmortem.

Autopsy: An ulcer at seat of injection similar to the one described for monkey No. 3. The inguinal gland near this region is as large as a hen's egg, and tuberculous. Lungs congested and thickly sprinkled with opaque, yellowish white areas, of a cheesy character, varying in size from points to 1 cm. or more diameter. Liver closely sprinkled with opaque yellowish white, necrotic areas, from points to 1 mm. diameter. Spleen greatly enlarged and mottled; closely studded with raised, opaque, yellowish white areas, from 1 mm. to 1 cm. or more diameter; these areas, like those in the lung, are filled with softened, cheesy, necrotic material. Omentum is sprinkled with minute white points. Lymph glands show lesions of tuberculosis. (See Pls. XXVII and XXVIII.)

Record of monkey No. 5.—Male rhesus, in excellent condition, tested with tuberculin without reaction; weight, 5 pounds 11½ ounces. November 7, 1901, injected, subcutaneously, inside right thigh, with 1 c. c. human tubercle culture, No. 50.

The condition of this monkey, relative to the lesions at seat of injection and general constitutional symptoms, is so much like that described for monkeys Nos. 3 and 4 that a separate description would be superfluous. The monkey died December 21, at which time he weighed 4 pounds 1 ounce.

Autopsy: Lesion at seat of injection similar to that found in monkeys Nos. 3 and 4. Lungs—The upper or anterior lobe on the right side completely solidified, the lower portion of the corresponding lobe on the left side in a similar condition; the lungs as a whole show a sprinkling of translucent, grayish white areas, from 1 to 2 mm. diameter. Liver contains numerous small, opaque, yellowish white areas, varying in size from mere points to areas 1 and 2 mm. in diameter. Spleen dark in color, and closely covered with elevated, yellowish white areas from 1 to 10 mm. diameter. Lymph glands show enlargement and necrotic changes. The gland in inguinal region contains thick, creamy pus. (See Pl. XXIX.)

Record of monkey No. 6.—Female rhesus, in excellent condition, tested with tuberculin without reaction; weight, 5 pounds. November 7, 1901, injected, subcutaneously, inside of right thigh, with 1 c. c. bovine tubercle culture III.

November 20, a large fluctuating abscess has formed at seat of injection. December 4, the monkey is very sick and so weak that she can move about the cage only with considerable effort; her respiration is accelerated and labored. When she was caught to be weighed the abscess at seat of injection broke and discharged about 4 c. c. of creamy pus in which firm masses of necrotic tissue were held in suspension. The skin over the abscess is reddened and that surrounding it is swollen. Weight, 3 pounds 15 ounces. December 11, found dead; weight, 3 pounds 9 ounces.

Autopsy: Lung—The upper lobe on left side completely solidified; sprinkled throughout the organ are a few grayish white areas, about 1 mm. diameter. Liver apparently enlarged, and closely studded with small, opaque white areas, varying in size from points to 1 mm. in diameter. Spleen enlarged and closely sprinkled with raised, opaque white areas, varying from points to 2 mm. in diameter. The mesenteric glands are enlarged and show occasional yellowish white, cheesy areas. (See Pl. XXX.)

Record of monkey No. 7.—Female baboon, received at the experiment station January 30, 1902; tested with tuberculin without reaction February 13, 1902. General condition very good.

This monkey was fed with milk obtained from three cows, as follows: From February 21 to 25, inclusive, the milk of a cow which had received an injection of bovine culture III a month previously into the connective tissue which binds the posterior half of the udder to the body; from February 26 to 30, inclusive, the milk of a cow supposed to have a naturally infected udder; from May 22 to July 9, inclusive, the mixed milk of the two above cows; and from July 17 to November 10, inclusive, the milk of a cow which had received, on July 9, an injection of bovine tubercle culture III into the udder through the teats.

Careful microscopic examination of the milk of the first two cows, also the injection of guinea pigs from time to time with this milk, failed to demonstrate the presence of tubercle bacilli; while similar tests of the milk of the third cow showed tubercle bacilli under the microscope and caused fatal generalized tuberculosis in all the guinea pigs injected. Guinea pigs were injected from the last cow on the following dates: July 18, 19, 21, 22, 23, 24, 25, 26, and 28, and August 4, 7, 11, and 14, one guinea pig each day, with the exception of July 19, on which day six were injected. Six guinea pigs were also fed milk from this cow from August 16 to November 10, on which date they were killed, and three of them were found to be affected with tuberculosis.

The monkey received milk twice daily, about 120 c. c. at each feeding. Her general record is as follows: From February, 1902, to January, 1903, condition excellent. Retested with tuberculin without reaction on April 18, 1902. Weight on February 28, 1902, 9 pounds 9 ounces; on April 9, 10 pounds 1 ounce; and on July 21, 11 pounds 2 ounces.

In January, 1903, about 6 months after the feeding of the milk of the third cow began, and about 2 months after it was stopped, a perceptible loss in condition occurred. January 30, 1903, respiration accelerated and accompanied by a wheezing sound. February 3, the weight had fallen to 10 pounds. February 18 acted as though affected with headache. February 28, has a discharge from nostrils and is drowsy. March 14, is very much weaker and lacks appetite; discharge from nostrils continues. March 24, is very sick and weak; sits on the floor of cage with head thrown back; has a profuse discharge from nose, and refuses all food. March 26, died late in the afternoon; weight, 6 pounds 14 ounces.

Autopsy: Extremely emaciated. Lungs—Sprinkled over the pulmonary pleura are a number of tubercles from 1 to 8 mm. diameter, which have a gray hue. Many similar tubercles on the costal pleura, on which are also found tubercles with pedicles which greatly resemble the appearance frequently found in pearl disease of cattle. Liver contains a few white, sharply outlined areas, about 2 mm. diameter, and a large number of small grayish points scattered over its entire surface. Spleen is enlarged and dark in color, and scattered through it are a large number of caseous areas. Careful examination of the intestine failed to reveal disease. Guinea-pig injections and microscopic examination of the various organs demonstrated the presence of tubercle bacilli. (See Pl. XXXI.)

TABLE VII.—*Monkey experiments.*

Material used.	Method of administration.	Dose.	No. of monkey.	Date of inoculation.	Result of inoculation.
		c. c.			
Culture bovine III	Subcutaneous	1	1	Sept. 12, 190	Killed Oct. 30, 1901. [a]
Do	do	½	2	do	Killed Nov. 22, 1901. [b]
Culture human No. 50	do	1	3	Nov. 7, 190	Killed Dec. 30, 1901. [b]
Culture bovine III	do	1	4	do	Killed Dec. 23, 1901. [b]
Culture human No. 50	do	1	5	do	Died Dec. 21, 1901. [c]
Culture bovine III	do	1	6	do	Died Dec. 11, 1901. [c]
Tuberculous milk (culture bovine III).	By mouth	([d])	7	[d]July 17, 1902	Died Mar. 26, 1903. [c]

[a] In dying condition when chloroformed. Generalized tuberculosis.
[b] In dying condition when chloroformed. Lesions of generalized tuberculosis.
[c] Lesions of generalized tuberculosis.
[d] Fed twice daily until November 10, 1902.

It will be well for us to state that the tuberculous character of the lesions in the monkeys used in the experiments was confirmed in all cases by microscopic examination or by guinea-pig injection, or by both.

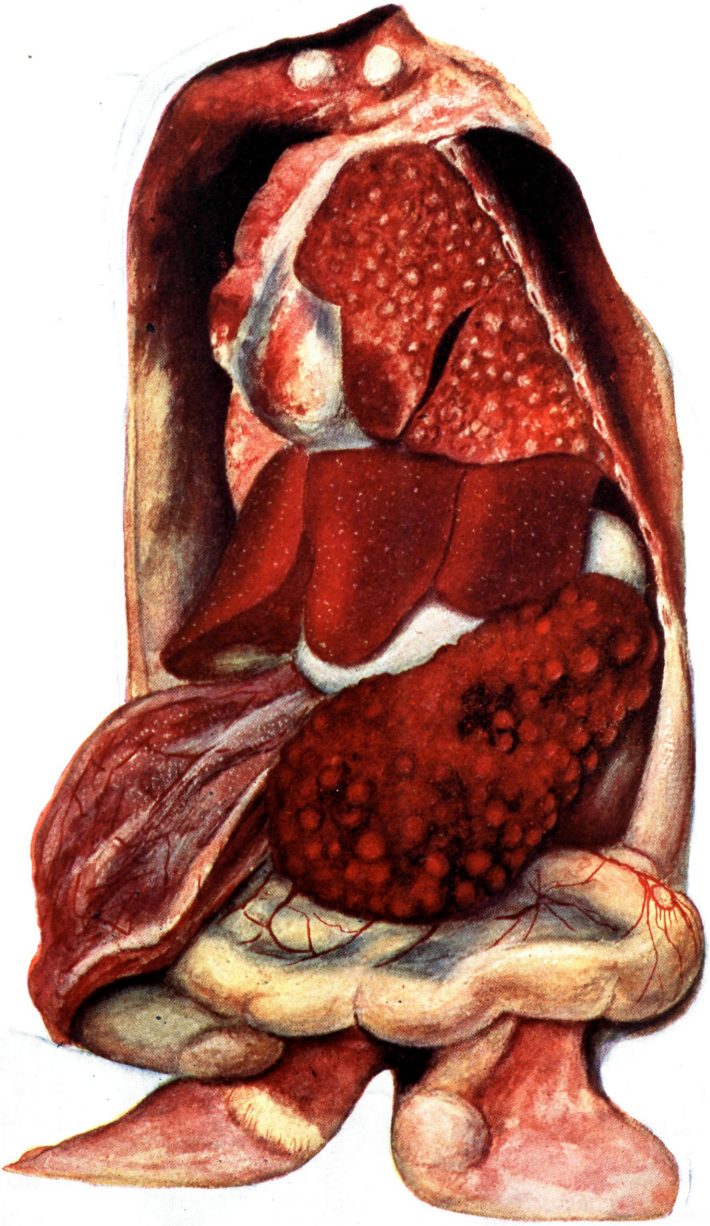
DESCRIPTION OF PLATE XXVIII.

Sections from monkey No. 4, indicating the distribution of the bacilli in the diseased organs resulting from subcutaneous inoculation with bovine culture III. Stained with hematoxylin and carbol-fuchsin. Magnified 1,450 diameters.

Fig. 1.—Section from liver.

Fig. 2.—Section from spleen.

Drawing by W. S. D. Haines.



Monkey No 4. Baboon,
subcutaneous inoculation with pure culture bovine tuberculosis No. III.

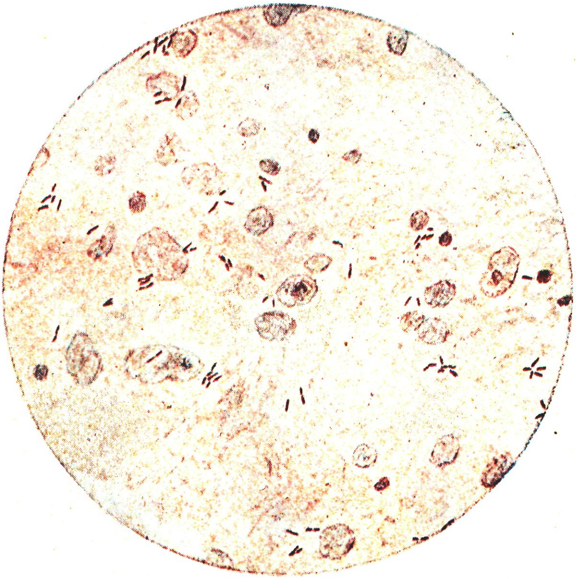


Fig. 1.

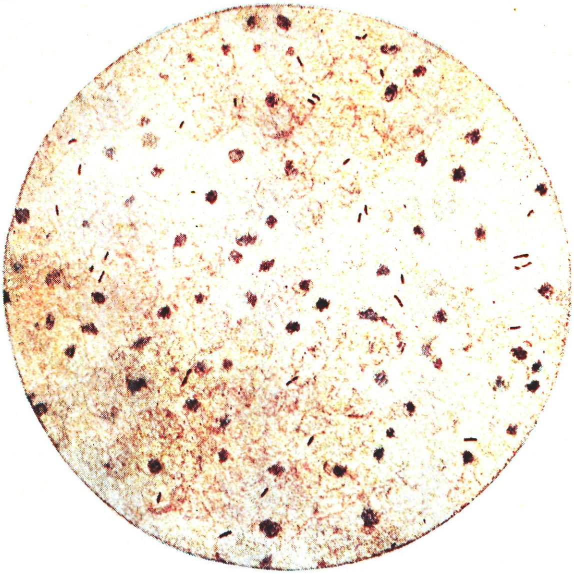
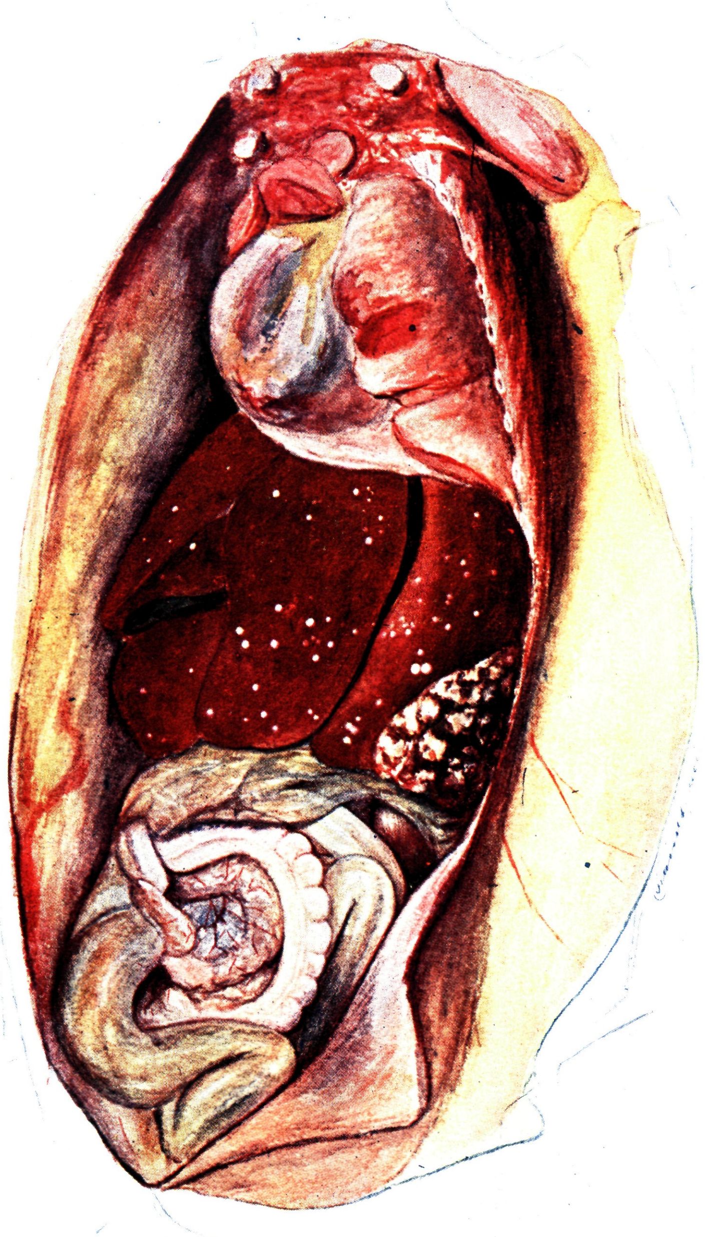
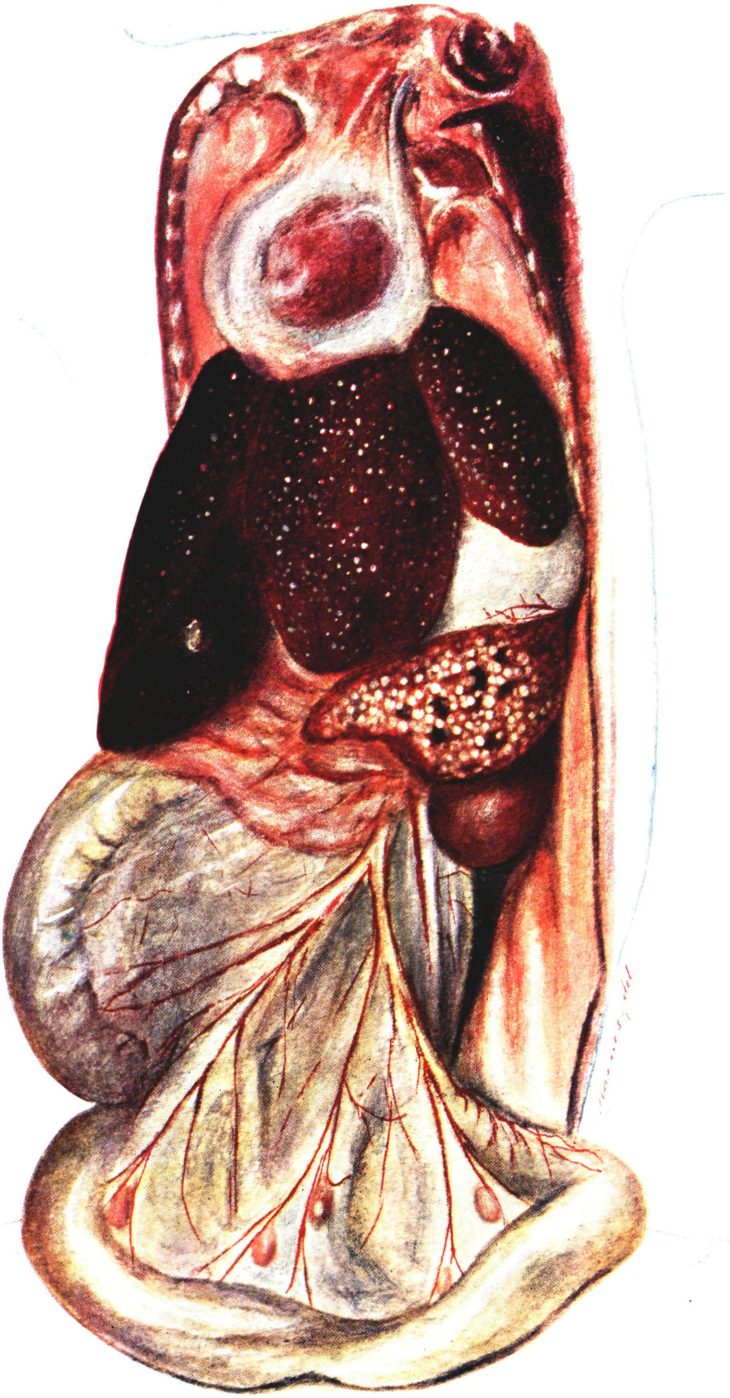


Fig. 2.

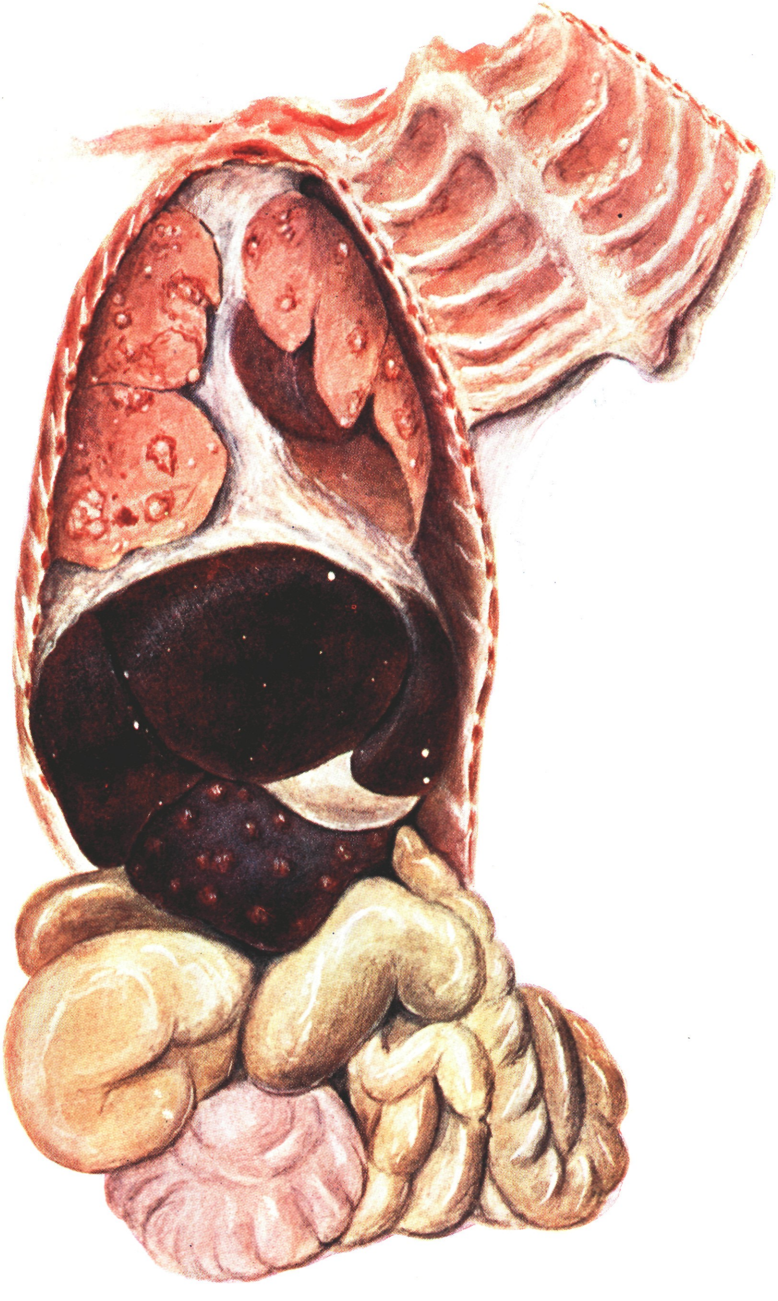
Tubercle bacilli in sections from Monkey No. 4. Bovine tuberculosis No. III.



Monkey No. 5. Rhesus.
Subcutaneous inoculation with pure culture human tuberculosis No. 50.



Monkey No. 6. Rhesus, subcutaneous inoculation with pure culture bovine tuberculosis No. III.



Monkey No. 7, Baboon. Fed on tuberculous milk. (Bovine tuberculosis.)

DISCUSSION OF MONKEY EXPERIMENTS.

The results obtained in the experiments with monkeys seem to justify the conclusion that no important difference exists between the two tubercle cultures used. One was a standard culture of human origin and the other a standard culture of bovine origin. An examination of the monkey records and of Plates XXV, XXVII, XXIX, XXX, and XXXI gives no other evidence against the identity of the two germs than that of a possible difference in virulence, and that a difference in virulence of tubercle bacilli from different sources exists, at times even when they are obtained from different organs of the same subject, is an old story.

As monkey No. 7 was kept under observation a long time, and gave no evidence of tuberculosis until a number of months had elapsed after she was fed milk actually containing tubercle bacilli, it may be well to say, in support of our conviction that the disease was due to the tubercle bacilli contained in the milk, that several other monkeys of the same species were kept under precisely similar general conditions, in other experiments, in adjoining cages in the same one-roomed house without contracting tuberculosis. This monkey indicates at one and the same time that tuberculosis contracted through ingestion progresses more slowly than tuberculosis induced by a subcutaneous injection; that a fairly generalized fatal tuberculosis can be produced by ingestion without specific lesions of the intestines; and, finally, that monkeys, notwithstanding their distance from cattle in the animal scale, are highly susceptible to the pathogenic properties of tubercle bacilli of bovine origin which gain entrance to their bodies with the food they eat. The question regarding the susceptibility of man to tubercle germs of bovine origin can not be more closely approached in an experimental way, and this experiment alone, supported by the large existing mass of circumstantial evidence of the infectiousness of tubercle bacilli of bovine origin for man, seems sufficient to warn every reasonable mind against the use of milk from tuberculous cows, and to point out with the greatest emphasis that it is desirable to take measures which will eventually eliminate all tuberculous cows from our dairy herds.

While it is not disputed that a majority of the persons who become infected with tuberculosis contract the disease from other human beings, it is maintained that cattle also constitute a very important source of infection. The importance of the latter source can not easily be overestimated in the light of the very considerable evidence which is accumulating in support of the view that human and bovine tuberculosis are not essentially dissimilar. It is true a difference does exist between certain tubercle bacilli of human origin and those of bovine origin, but it is not a specific difference or even a difference which permits the grouping of the germs as distinct varieties of the same species, but a difference practically confined to pathogenic virulence, and which forces us to regard the tubercle bacilli of bovine origin merely as more virulent than those of human origin.

REVIEW OF SOME OF THE MORE RECENT EXPERIMENTS CONCERNING THE INTERTRANSMISSIBILITY OF HUMAN AND BOVINE TUBERCULOSIS.

The well-known address of Prof. Robert Koch[1][a] before the British Congress on Tuberculosis in July, 1901, caused an enormous awakening of interest among medical men in all that concerned the relationship of human to bovine tubercle bacilli. In the paper referred to Koch expressed the belief that the infection of man with tuberculosis from bovine sources is of such rare occurrence that no preventive measures are necessary in this direction. He was led to this belief by the lack of direct evidence that such infection had taken place, and by the experiments conducted by himself and Schütz,[2] in which it was found that a number of tubercle cultures from human sputum would not infect cattle or certain other domesticated animals. This London address led medical investigators the world over to make experiments and observations bearing more or less directly on the intertransmissibility of human and bovine tuberculosis. By the fall of 1902 a large amount of evidence had been presented to show that certain tubercle bacilli of human origin are capable of causing a generalized tuberculosis in cattle, and many instances of the infection of men from cattle had been reported. These reports, which possibly modified Professor Koch's views in regard to the pathogenic power of human tubercle bacilli for cattle, did not influence his opinion in regard to the danger to human beings from the use of tuberculous meat and milk, as is shown in his latest paper[3] upon the subject. His views, as expressed in this paper, present the strongest possible statement of the position occupied by those who insist upon the noninfectiousness of bovine tuberculous virus for human beings. For that reason it seems desirable to quote in part the requirements which, in his opinion, should be met in order to establish the infectiousness of bovine tuberculosis for the human subject. The requirements are in part as follows:

1. Certain proof of tubercle in general and, where possible, the primary focus must be supplied. In adults we must, therefore, require the existence of unassailable clinical symptoms, and a necropsy when these are not present. In children the clinical symptoms are much too uncertain, and so a necropsy is always requisite with them.

2. Other sources of infection must be excluded with certainty. The assurance that the person in question comes of a healthy family is under no circumstances sufficient. There

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[a] These figures refer to bibliography on page 98.

are numerous other possibilities of infection either inside or outside the family which come up for consideration. * * *

3. In each case of alleged infection from milk infected with perlsucht bacilli the condition of the rest of the people who have taken the same milk should be borne in mind. These fellow-consumers form to a certain extent a control experiment, and if of the numerous people who have drunk the suspected milk only a single one sickens, this weighs decidedly against the belief that this one person was infected by the common food. * * *

4. The source of the milk should be attended to. Since in recent years it has become more and more evident that milk containing tubercle bacilli is yielded only by cows affected with tuberculosis of the udders, the general statement that someone has drunk milk from a cow suffering from perlsucht no longer suffices to prove to us that perlsucht bacilli have really reached that person's organs of digestion. A man can certainly ingest milk from a cow suffering from perlsucht without coming into contact with perlsucht bacilli through it. It must be milk from a cow with tuberculosis of the udder, and therefore a statement relative to this point should not be lacking in a report on milk infection if it is to be considered complete.

It will be seen from the above statement that Doctor Koch requires absolute and direct proof of the infection of men with bovine tuberculous virus before he will recede from his position that there is practically no danger to human beings from tuberculous meat and milk. We are inclined to regard some of the conditions imposed by him as unnecessary and unreasonable because, owing to the insidious onset of this disease, and the fact generally admitted that tubercle bacilli which have entered the body may lie dormant for years without producing any evidence of their presence, it is impossible to say with absolute certainty when or where any given case of human tuberculosis had its origin. We are, therefore, forced to rely almost wholly upon indirect evidence as to the infectiousness of bovine tuberculosis for men. In our opinion the intertransmissibility of human and bovine tuberculosis is supported by so much indirect evidence of an overwhelming character that we are justified in regarding it as practically established. Absolute proof is greatly to be desired, but in its absence we must be governed by probabilities and base our regulations for the control of tuberculosis on them.

In the following pages are reviewed some of the most recent experiments bearing upon human and bovine tuberculosis, together with a portion of the literature concerning the transmission of tuberculosis to animals by feeding.

EXPERIMENTS WHERE INFECTION WAS BY MEANS OF DIGESTIVE TRACT.

Dobroklonski[4] has attempted to decide definitely the disputed questions whether tubercle bacilli can enter the body through the intestinal tract and whether a lesion of the intestinal mucous membrane is necessary for such infection to take place. The animals experimented upon were guinea pigs. The infecting material used consisted of tubercle cultures on glycerinized bouillon. A small glass tube with rounded ends was inserted into the throats of the guinea pigs and a few drops of a pure culture were allowed to flow through it. The animals were not fed for some little time after they received the culture and were guarded carefully against any outside infection. They were killed on the fourth, tenth, fifteenth, twentieth, twenty-fifth, thirty-second, and fortieth days, respectively, after being fed the culture. At the autopsy a careful macroscopic examination was made of the entire length of the intestine, and microscopic sections were made from large portions of that organ, those portions which indicated the slightest variation from the normal being given specially careful study. As a result of this extremely laborious experiment the author found that it is entirely possible for tubercle bacilli to pass through the normal intestinal mucosa of guinea pigs. He is not able to say just how the entrance is effected, but draws the following conclusions:

Tuberculosis may certainly infect animals through the digestive tract. For this infection to take place, no lesion of the intestinal wall is necessary; no epithelial desquamation; no local changes of any character; no previous inflammatory process. The tuberculous virus—the bacilli as well as the spores (sic)—can easily pass through the epithelial covering of the perfectly normal intestine. This penetration is particularly easy in cases where the contact of the tuberculous virus with the intestinal wall is prolonged, but for the penetration to take place it is not necessary for this contact to be prolonged. The tubercle bacilli as well as their spores (sic), if they are not long in contact with the intestinal wall, are not capable of provoking inflammatory processes or modifications of the epithelial lining of this wall. The tubercle bacilli probably have no definite way of gaining entrance into the organism—for example, by leucocytes exclusively—but they may be carried by the lymph current and later being arrested at some places in the tissues they there give rise to the formation of tubercles.

Nocard [5] says that in the case of cats, young animals are especially liable to contract tuberculosis through the digestive tract. He fed to four 6-weeks-old kittens and also to their mother a paste with which was mixed a very abundant culture of tubercle bacilli. All four kittens died within four months of a severe type of tuberculosis. The mother lived for a long time in good health, but when killed at the end of three years tuberculous lesions of the spleen, liver, and lung were found.

Clausen,[6] while holding the position of slaughterhouse inspector in the town of Husum, in Germany, was struck by the fact that the hogs slaughtered for the almshouse and hospital were more or less infected with tuberculosis. Hogs of the same breed fed in other places, however, were entirely sound. The tuberculous changes were usually found in the liver, mesenteric glands, cervical glands, or the lungs. Hogs suffering from generalized tuberculosis had to be condemned as unfit for human food. On this account it became very desirable to find the cause of this disease. As the hogs at the almshouse were supposed to be fed only upon barley, corn meal, cooked potatoes, and some green fodder, the food was not at first suspected. The stalls were in very good condition; nevertheless, before the introduction of fresh hogs they were cleansed with hot soda solution and disinfected with carbolic acid, and fresh lime was scattered over them. One day, quite accidentally, Clausen noticed that the refuse from the hospital portion of the institution, in which a number of tuberculous patients were being cared for, was thrown out to the hogs. He forbade the further continuance of this practice, and never found another tuberculous hog at that institution. As a result of this observation the author is convinced that hogs may contract tuberculosis directly from men. He consulted his day-book and found that hogs which had been cared for by men suffering with tuberculosis often showed that disease when slaughtered.

Tjaden[7] reviews the statistics concerning primary intestinal tuberculosis in children and also reports some experiments conducted by himself in collaboration with Hertel and Koske. They fed milk from tuberculous cows to sixteen young pigs. Only one of these developed a primary intestinal tuberculosis. The others had an infection of the mesenteric and also of the cervical lymph glands. From this fact the author infers that a great number of the cases of tuberculosis of the cervical glands in children may be caused by tuberculous milk, and that infection through the food is not always indicated by tuberculosis of the intestines.

Nocard[8] used six monkeys of the same origin for an experiment, which was briefly as follows: Three of the monkeys were reserved as checks. They remained perfectly healthy. The other three, which were fed several rations containing a small amount of a culture of bovine tubercle bacilli, became tuberculous after a short time. The localization of the lesions thus produced showed clearly that the intestinal mucous membrane was the entrance point of the virus, and there can be no doubt that the infection was the result of eating the infected food. The monkeys showed the same susceptibility to bovine tuberculosis that is seen in all other mammals; therefore, in Nocard's opinion, we have no right to suppose that there is a difference in the case of children. The milk from a cow suffering with udder tuberculosis must be considered as especially dangerous.

Cipollina[9] records the results obtained by feeding an ape with cow's milk, to which had been added a pure culture of bovine tubercle bacilli in the proportion of 1 öse to 150 c. c. of milk. This feeding was kept up for a month, the tubercle bacilli being administered every other day. Before the feeding commenced the animal was kept under observation for some time and carefully watched in order to see if it showed any signs of illness, and it was also tested with tuberculin before the experiment began, the test giving a negative result. The animal began to lose in weight and show symptoms of illness after a month and a half and died at the end of three months. The autopsy showed a severe tuberculosis of the abdominal organs and many nodules in the omentum and over the serous surface of the intestines. The mucous membrane of the stomach and intestine was not affected, with the exception of a few small areas in the colon, which were slightly hemorrhagic. This condition was due to the intestine being adherent to diseased mesenteric glands at those points. The kidneys and lungs exhibited many caseous nodules. This experiment, according to the author, shows, first, that the ape (Bertuccia) is susceptible to cattle tuberculosis by feeding; second, that the bovine tubercle bacillus may pass through the intestine without leaving any lesions at the point of entry.

Ravenel[10] has reported a very interesting experiment along the same line as those conducted by Dobroklonski, Tjaden, and Cippollina. Healthy dogs, after receiving a dose of castor oil, were fasted for twenty-four hours and then fed a single dose of melted butter and warm water containing a large number of tubercle bacilli. Three and one-half to four hours after feeding the dogs were killed and as much chyle as possible collected, together with the mesenteric glands. With this material guinea pigs were inoculated and microscopic examinations were also made. The entire intestine was carefully examined, but in no instance could a lesion be found. Of the ten dogs submitted to this treatment eight gave positive results. The author attributes the two failures to the fact that the tubercle bacillus used for feeding was very low in virulence, having been cultivated in the laboratory for a number of years. As a result of this experiment Ravenel concludes that—

> Under certain conditions tubercle bacilli pass through the normal intestinal wall with great facility and rapidity. The most favorable condition for this to take place appears to be during the digestion of food made up largely of fat. When we remember that the chyle is carried directly into the blood stream through the thoracic duct it is easy to understand how it is that infection through food may show itself first in the lungs, or at any rate that the lesion in the lung may be as old as the intestinal lesion. The claim that a food tuberculosis should show itself in a primary intestinal lesion is fallacious and misleading.

Schottelius[11] used three calves and two cows for a feeding experiment and reserved one cow and calf for control animals. The animals came from a mountainous region where tuberculosis did not exist. The calves were six weeks old and were sound and healthy. The infectious material was sputum from a tuberculous patient. The sputum was fed to the calves with milk and to the cow with green fodder. Each animal received about 50 grams at a feeding. From the 25th of May to the 29th of August 24 such feedings were given. There were no characteristic symptoms of illness. On the 20th of September the animals were slaughtered. Both control animals were perfectly healthy. The postmortem showed the following lesions in the three inoculated animals: In the cow, tuberculous enteritis, considerable swelling of the mesenteric glands, tuberculous caseation and calcification of the mediastinal and bronchial glands, and a caseous tuberculous pneumonia, with a few miliary tubercles on the pleura. In both calves there were greatly swollen, tuberculous, caseous, and calcified submaxillary glands and a few tuberculous mesenteric glands. The microscopic and bacteriologic examinations showed the presence of tubercle bacilli in the diseased parts.

Hüls[12] reports from his early practice the family of a miller, in which no case of tuberculosis had occurred until, within five years, seven members—six adults and a 13-year-old child—sickened and died of the affection. The beginning of the tuberculosis Hüls traces to the fact that a few years before the family became infected a strange cow, suffering with tuberculosis, was purchased, and the miller's entire herd of cows became infected through the entrance of this new animal. The incident is recognized by the author as not proving the identity of human and bovine tuberculosis, but, taken together with other observations, points strongly to the possibility of their intercommunicability.

Heller[13] agrees with Koch that hereditary tuberculosis does occur, but is so rare that it can be left out of practical consideration. He disagrees with Koch as to the infectiousness of bovine tuberculosis for man, and concludes from the examination of a large amount of material that primary infection of the intestinal tract occurs with a considerable degree of frequency. This investigator recognized how difficult it is to obtain data regarding the original center of disease by the examination of tuberculous cadavers because of the advanced character of the lesions and therefore used the material from diphtheria autopsies, as it is well known that tuberculosis frequently follows diphtheria. Among the 714 autopsies made, 140, or 19.6 per cent, showed the presence of tuberculosis. Of the latter, 43, a small fraction less than 31 per cent, showed primary tuberculosis of the intestinal lymph glands, and 10 additional cases showed disease of the abdominal organs associated with lung affection.

In the presence of abdominal tuberculosis in children the food must be suspected, and of this cow's milk is the most important. The objection that children lie on the floor a great deal and put all manner of things into their mouths, and contract intestinal tuberculosis in this way, does not explain why children rarely suffer from pulmonary tuberculosis, to which they are equally exposed by their habit of crawling, which facilitates the breathing of dust charged with tubercle bacilli.

Heller shows the infectiousness of bovine tuberculosis for man in a very striking and well-investigated case, in which a man who pricked milk into his skin in order to remove tattoo marks developed lupus at the point of inoculation. On the ground of these investigations Heller strongly opposes a relaxation of the former regulations for the control of bovine tuberculosis.

INOCULATION EXPERIMENTS.

Cipollina[9] describes the inoculation of a calf with a culture of human tuberculosis; details not given. The inoculation was made in the following manner: The animal was allowed to inhale tubercle bacilli through an opening in the trachea, and also received an intraperitoneal injection of 10 c. c. of the same culture. After twenty days the animal was well and was inoculated for the second time intraperitoneally with 20 c. c. of the same culture. The animal died after two months, but showed no signs of tuberculosis. The author concludes that cattle are very resistant to human bacilli, but in the light of researches upon ordinary experimental animals he believes that the human bacillus may infect all animals that are susceptible to the bovine bacillus, the only difference being that the latter is almost always more virulent than the former. This explains why cattle can resist human tuberculosis and succumb to bovine tuberculosis, and what is true of other animals is probably also true of man. The bacillus of bovine tuberculosis is, therefore, more virulent for man than the human bacillus itself.

Bang[14] noted the following in regard to his experiments upon the transmission of human tuberculosis to cattle. In two instances human tuberculosis was inoculated into the anterior chamber of the eye of calves with positive results. In the first case the material was taken from a tuberculous human lung. A guinea pig was inoculated with this material and the spleen of this guinea pig furnished material for the inoculation in both eyes of an 8-weeks-old calf. The primary inflammation was unimportant and quickly disappeared, but about eighteen days later a severe tuberculous iritis and conjunctivitis developed. After about seven weeks the calf was killed. At the autopsy both eyes showed a distinct tuberculous iritis. The other organs of the body, however, exhibited no changes. In the second case Bang injected into the anterior chamber of the eye of an 8-weeks-old calf some pus which was obtained from the tuberculous lymph gland of a child. The result was quite similar to that in the first case. The pus was quickly absorbed and a week after the inoculation the eye was apparently in a normal condition. Three weeks after the inoculation a tuberculous iritis developed and was followed by a keratitis and a conjunctivitis. Four and one-half months after the inoculation the iris was studded with white nodules.

Both of these cases show that human tuberculosis is communicable to cattle. They indicate also, however, that human tuberculosis pursues a slower and more benign course in cattle than the true bovine tuberculosis.

Orth[15] reports the following experiments: Three calves, three hogs, and three goats were used. The animals were all obtained from yards which were known to be free from infection, and the calves gave a negative reaction to the tuberculin test shortly before the commencement of the experiments. The infectious material was obtained from a case of phthisis cavernosa by inoculating a guinea pig and then obtaining cultures from this animal upon brain and other media. In the experiments bouillon cultures of the organisms were used and in some cases tuberculous organs from rabbits which had been injected with such cultures.

Of the three calves, the first one, which received a bouillon culture, injected into the lung through the trachea, gave an entirely negative result. The second, which was inoculated between the abdominal muscles with a suspension of the pure culture, showed only a local caseous abscess. Both animals were killed five and one-half months after the beginning of the experiment. The third calf, which was inoculated in the peritoneal cavity with two pieces of tuberculous kidney from a rabbit, died on the twenty-sixth day of a general tuberculous peritonitis. The serous membranes were covered with miliary and submiliary tubercles. The great omentum, which was adherent to the peritoneum, was sprinkled throughout with nodules. Small nodules were visible on the serous membrane of the intestines. The mesenteric, portal, and cardiac lymph glands were much enlarged and showed grayish-white nodules upon section. The retrosternal lymph glands were much enlarged and congested, and small nodules were visible upon section. The left shoulder gland and other lymph glands were swollen and some exhibited tuberculous changes. The other organs were free from tuberculosis. The microscopic examination of the peritoneal nodules and the various lymph glands, especially the retrosternal and submaxillary glands, showed typical tuberculous granulations containing giant cells and many tubercle bacilli, which were especially abundant in the giant cells.

The guinea pig inoculated from this calf died within a few days, so that this experiment could not be carried further. It can be said, however, that human tuberculosis has been communicated to a calf, and not simply a local disease, but a progressive, fatal tuberculosis was produced. There can be no doubt that this calf was infected with human tuberculosis, for in the author's various experimental animals no case of spontaneous tuberculosis had developed and no bovine inoculations had been made. The condition of the calf could not be ascribed to the action of dead bacilli, for the bacilli with which the calf was inoculated were inclosed in a tuberculous kidney tissue and must have spread over the entire peritoneum and also to portions of the body far removed from the point of inoculation.

In the case of the three hogs, the two which were inoculated intraperitoneally with the bouillon culture gave practically negative results. The third hog was inoculated through the trachea into the lungs with a bouillon culture. After five and one-half months the animal was killed. In the thymus gland and in the membranes around it there were many yellowish tuberculous nodules. The left lung was free from tuberculosis, but in the inferior lobe of the right lung there were found a large number of submiliary and miliary tubercles. No other tuberculous changes were found. The microscopic examination of the nodules presented a typical picture of tuberculous granulations containing giant cells and exhibiting a central caseation. Tubercle bacilli were present.

The three goats all showed tuberculosis following inoculation, though it is worthy of note that the two most marked cases resulted from inoculation with the tuberculous organs of a rabbit. The first animal, which was inoculated with a bouillon culture through the trachea into the lung, showed a number of subcutaneous nodules near the point of inoculation, a large caseous nodule in the thymus gland and a number of yellowish gray nodules in the inferior lobes of the right and left lungs. No lesions were found in the abdominal viscera except a few yellowish nodules in the liver, which, upon microscopic examination, were found to be tuberculous in character.

The second goat was inoculated intraperitoneally with a small piece of lung from a tuberculous rabbit. The animal was killed after five months and showed the following lesions: A number of nodules in the muscles and subcutaneous tissue at the point of inoculation. A number of small grayish, translucent nodules were found scattered through the omentum. They were found especially in the portion near the stomach and some of them hung by small pedicles resembling polypi. Similar changes were found in smaller number on the diaphragm and in the neighborhood of the lymph glands surrounding the heart. On the pleural surface of the diaphragm these nodules occurred singly. On the anterior border of the liver near the gall bladder there was found a nodule about the size of a pea. Microscopic examination showed the nodules to be tuberculous granulations containing giant cells with a necrotic center and often lime salts were present. Bacilli were found, although in small numbers.

The greatest changes were found in the third goat, which was inoculated with the same tuberculous kidney with which calf No. 3 was inoculated. A fragment about the size of a bean was introduced into the peritoneal cavity of the goat. The animal was killed at the end of five and one-half months. A number of tubercles were found around the point of inoculation. There was a large group of caseous nodules on the adjoining surface of the stomach, numerous caseous nodules in the omentum, many of these latter having small pedicles. There were some nodules and granulation tissue attached to the capsule of the liver. The interior of this organ, however, was free from tuberculosis. The retroperitoneal lymph glands were very large but not caseous. All other organs appeared to be normal. Nodules from the various diseased areas were examined microscopically and showed tuberculous new formations with giant cells, central necrosis, and calcification. Tubercle bacilli were easily demonstrated. There can be no doubt as to the tuberculous character of the lesions described nor that they were produced by human tubercle bacilli. Therefore the author takes issue with Koch's statement that tuberculosis of man can not be communicated to cattle.

To answer the question as to whether he has really obtained a perlsucht or simply a tuberculosis different from that of cattle, the author points out that in the third goat the abdominal lesions were entirely of the perlsucht character. He also points to the case reported by Baumgarten in which by injection of perlsucht bacilli into cattle, a generalized miliary tuberculosis was produced without any resemblance to the typical perlsucht. The morphological changes can not be relied upon to differentiate between human and bovine tuberculosis, because these changes depend upon the condition of the animal as well as upon the organism itself. How little the perlsucht form of a tuberculous disease is dependent upon the perlsucht bacillus has been shown by Troje and Tangl who, by subjecting human tubercle bacilli to the vapor of iodoform, succeeded in producing a typical perlsucht in rabbits.

Orth concludes that it is proven that human tuberculosis can be communicated to cattle. It must be remembered also that the danger of the communication of bovine tuberculosis depends not only upon the transmission of bovine bacilli, but also upon the constitution of the person who is exposed to them.

Fibiger and Jensen[16] report the results of their experiments with human tuberculous material obtained from the various hospitals of Copenhagen. They report in all five cases of tuberculosis in the following subjects: (1) 42-year-old woman, (2) 11-year-old girl, (3) 6-year-old boy, (4) 19-months-old girl, and (5) 4-months-old boy. In all of these cases the infection appears to have begun in the intestines and in some the disease was practically confined to the abdominal organs.

(1) A 3-months-old calf was inoculated in the thoracic cavity with a suspension made from a mesenteric gland of case No. 1. This calf

remained apparently in good condition and was killed at the end of six months. Two miliary tubercles were found on the pleura and a few fresh pearl nodules were found on the pulmonary pleura; otherwise the animal was in good health.

(2) A 3-months-old calf which had been subjected to the tuberculin test was inoculated intraperitoneally with a suspension made from the spleen of a guinea pig which had been inoculated with a mesenteric gland from case No. 2. The health of the calf was not visibly affected by the inoculation. It was killed at the end of the fifth month. In the omentum was found a large number of small red growths and about twenty small, fresh, pearl nodules. On the diaphragm, also, there were some small nodules and excrescences; on the pulmonary pleura a few excrescences.

(3) A calf 2 to 3 months old which had withstood the tuberculin test was inoculated intraperitoneally with a suspension made from the spleen of a guinea pig which had been inoculated from case No. 3. There were no acute symptoms following the inoculation, although at the end of three weeks and later there was a slight rise in temperature. The calf was killed about the end of the fifth month. The omentum was filled with tuberculous new growths and showed countless small nodules of varying size. All lesions were recent; a few showed traces of caseation. There were some small nodules found on other portions of the peritoneum. The surface of the spleen and liver was covered with small pearl nodules, as was the diaphragm. Some of the mesenteric glands were enlarged. In the lungs a few small areas of atalectasis; no tuberculosis. The bronchial and posterior mediastinal glands were enlarged but showed no caseation.

(4) A calf 2 to 3 months old which had been subjected to the tuberculin test was inoculated subcutaneously on the right side of the neck with a suspension made from the mesenteric gland of case No. 3. The animal showed no very marked symptoms following the inoculation and was killed at the end of the seventh month. There was a large caseous mass at point of inoculation. The neighboring glands were enlarged, caseous, and calcified. The lungs had scattered through them many small miliary nodules and there were a number of excrescences attached to the pulmonary pleura. No caseation in these excrescences. The bronchial and posterior mediastinal glands were enlarged, hard, and dotted with fresh caseous and calcified nodules. The liver and spleen had scattered through them miliary tubercles. The omentum was filled with fine, diffused, red, new growths. In the mesenteric glands there was no evidence of tuberculosis.

(5) Two 10-days-old calves were inoculated subcutaneously on the side of the neck with a suspension made from a mesenteric gland

from case No. 5. Both of these animals died within three weeks of the inoculation and evidently from secondary infection. Although the calves died so soon there developed, nevertheless, a considerable local tuberculosis rich in bacilli

From calf No. 2, case 5, a third calf was inoculated and from calf No. 1 an old cow was inoculated. Calf No. 3, inoculated from calf No. 2, was fourteen days old and was injected subcutaneously in the right side of the neck. A large abscess formed at the point of injection. At the end of about two months the calf, being in a dying condition, was killed. The autopsy showed, in addition to the local lesions, tuberculosis of the adjacent lymph glands. There were many new growths on the costal and pulmonary pleura and also on the pulmonary surface of the diaphragm. The lungs contained a countless number of minute nodules, some of which were as large as hemp seed. The bronchial and mediastinal glands were enlarged and caseous. The liver, spleen, and kidneys had miliary tubercles scattered through them. The intestinal wall was very rich in fresh nodules and small superficial ulcerations. The mesenteric glands were enlarged and tuberculous. The omentum was rich in small new growths.

A cow about 10 years old, inoculated on the left side of the neck with material from calf No. 1, developed a phlegmonous abscess at the point of inoculation which was accompanied by fever, and tubercle bacilli were found in the pus. At the end of four months the cow was killed. In addition to the local abscess the neighboring glands were enlarged, caseous, and calcified. Miliary tubercles were scattered through the lungs. In the posterior mediastinal gland a nodule was found the size of a pea; in the liver, a small number of miliary tubercles. The other organs were normal.

Thus, in three cases of tuberculosis in children the bacilli present were virulent, some of them in the highest degree, for calves, and it is probable that the disease in the children was caused by bacilli which came from cattle. In the five cases the greatest variation in virulence for calves was found, ranging from nothing to the highest degree, and it is especially noteworthy that the virulence of these bacilli seemed to decrease as the age of the patient increased. Koch claimed that one could distinguish between human and bovine tuberculosis by inoculating calves subcutaneously. If this is true, then three of the cases above cited must be considered as perlsucht, and the assertion that tuberculosis of cattle is not virulent for men is disproved.

Karliński[17] succeeded in communicating tuberculosis to cattle by means of human tuberculous virus ten times in twenty-three attempts. The mode of inoculation varied. Some of the animals were inoculated intraperitoneally, some intrapleurally, some intratracheally, some into the udder, and some subcutaneously. He compared the cultures of human and bovine tubercle bacilli, but found absolutely no difference between them, either microscopically or macroscopically, except in the rapidity of growth. He considers that the chief difference is the greater resistance of cattle to human than to bovine bacilli. He has attempted to increase the virulence of the human tubercle bacillus by passing it through calves. With this object in view he inoculated one calf subcutaneously and intraperitoneally at the same time. At the end of two months the animal showed a distinct tuberculin reaction and was killed, and tuberculous changes were found in the mesenteric glands, liver, and spleen; some of the lesions showed a caseous degeneration. He recovered a culture from this animal and inoculated a second calf. This calf showed a tuberculin reaction in less than a month, but was allowed to live until the end of two months, when it was killed and showed a much more generalized tuberculosis than was found in the first animal, thus indicating that this particular tubercle culture had been increased in virulence by its passage through a calf.

Thomassen,[18] in the spring of 1899, tested two calves, aged 8 and 4 weeks, with tuberculin, obtaining negative results. The milk with which these animals were fed was taken from a cow which did not react to tuberculin. The 8-weeks-old calf received intraabdominally a culture upon glycerinized potato which was derived from the cerebral exudate of a child affected with tubercular meningitis. The animal remained well and was tested with tuberculin after two months and gave a good reaction. When the animal was killed, however, one month later, notwithstanding a most careful postmortem examination, no trace of tuberculosis was found. The calf 4 weeks old was inoculated in the anterior chamber of the eye with a culture of tuberculosis upon glycerinized potato which was derived from a case of tuberculous arthritis. After about a month this animal was tested with tuberculin, but failed to react. It was killed a short time afterwards and found to be affected with a generalized tuberculosis. The progress of the infection from the eye to the lungs was easily traced through the chain of lymphatic glands.

Some months later a cow about 2 years old, in perfect health, was tested with tuberculin and did not react. Shortly afterwards this animal was injected in the trachea with a culture of tuberculosis grown upon potato. This culture was obtained from a case of human renal tuberculosis. After two months a tuberculin injection failed to produce reaction. A few days after this second tuberculin test a culture was injected into the thorax and into the abdominal cavity. In making the abdominal injection some of the material was deposited in the muscular tissue. As a result of this an abscess containing purulent material formed and was found to contain tubercle bacilli. After five months the animal was tested with tuberculin without reaction. About ten months after this the animal was killed and showed not the slightest trace of tuberculosis. A fourth cow, 2 years old, was tested with tuberculin without reaction, and a culture of tuberculosis obtained from a case of renal disease in a man was injected into the anterior chamber of the eye. This animal after two months gave a marked tuberculin reaction, and was in very bad health. Six months after the inoculation the animal was killed. There were tuberculous lesions in the eye and in one bronchial gland, but unfortunately a histological examination could not be made. Thomassen's experiments show that it is difficult, but not impossible, to produce tuberculosis in cattle by means of cultures of the human bacillus. He thinks the much greater virulence of the bovine bacillus, which is generally admitted, should urge, contrary to the advice of Koch, a most rigid inspection of all meat and milk.

Arloing[19] inoculated thirty-three animals of various species with bacilli of human origin. The results were positive in every instance. Five separate cultures of human tuberculosis were used, cattle, sheep, goats, donkeys, and hogs were infected by intravenous injections and exhibited tuberculous lesions of different degrees. The human cultures varied greatly in virulence, but microscopic examination demonstrated that even the weakest had produced true tuberculous lesions. Compared with injections of bovine virus it can be said that, as a rule, the bovine virus is more virulent than the human, as all of the animals inoculated with bovine tuberculosis died within forty-four days, at the most, and the majority of those inoculated with human bacilli were killed. Arloing concludes:

> (1) Human tuberculosis is infectious for cattle, and at times, by certain methods of inoculation, it brings about lesions having the character of bovine tuberculosis.
>
> (2) The human bacillus is not always of the same virulence and does not show the same degree of activity toward different species of herbivora.
>
> (3) In some cases human bacilli are as virulent as germs of bovine origin. In other instances, on the contrary, the virulence is so reduced that it appears to be nil, especially for the bovine species.
>
> (4) Human bacilli of attenuated virulence always produce in the lung at least, following intravenous injections, lesions which are visible with the microscope.
>
> (5) The variations in the virulence of the bacillus account for the apparent negative results which have led Messrs. Koch and Schütz to believe in the duality of species. The unity of human and bovine tuberculosis should be maintained, and prophylactic measures which arise therefrom should be continued, especially with regard to the use of milk.

Ravenel[20] describes two cultures of the tubercle bacillus obtained from the mesenteric glands of two children. One (culture B B) he found to be quite as virulent for calves upon intravenous inoculation as the bovine bacillus, and the other (culture U) had a virulence decidedly above that of the ordinary human cultures. Culture

B B corresponded morphologically and culturally more nearly to the bovine type than to the human. He also reports the results of another experiment made by Dr. Leonard Pearson, in which a calf received large amounts (925 c. c. in all) of a human tubercle culture of moderate virulence at intervals extending over a period of four months, in the form of subcutaneous, intravenous, intrapulmonary, and intraperitoneal injections. This culture after successive passages through calves, showed a great increase in virulence.

Stenström[21] records experiments upon calves of reaction-free cows which were raised on boiled milk. Eight of these calves were inoculated in various ways with tuberculous sputum which was rich in tubercle bacilli. Three of the calves died shortly after inoculation and were left out of count. The remaining five were slaughtered six months after inoculation, and the autopsies showed the following results: Calf No. 820, inoculated into the lung, showed many tuberculous adhesions upon the costal and diaphragmatic pleura, as well as tuberculous areas in the mediastinal glands. Calf No. 833, infected by intratracheal injections, showed partly calcified areas in the anterior and posterior mediastinal glands and in the bronchial glands. Calf No. 224, inoculated intraperitoneally, showed many calcified areas in the omentum and enlargements of the glands within it, a fibrous exudate on the serous membrane of the peritoneal cavity, many tuberculous areas in the liver the size of a pinhead, and some adhesions on the surface of the right lung. Calf No. 94 had pulverized sputum sprayed into the nose and showed only slight inflammation of the pulmonary pleura. Calf No. 818, inoculated subcutaneously, showed only a large abscess at the point of injection and a proliferation of tissue around this abscess.

These results differ from those of Koch. None of Koch's nineteen calves showed any tuberculosis of the internal organs after six or eight months; only a small pus area containing a few tubercle bacilli was found. Stenström found in his animals that the tuberculosis spread from the point of infection to the internal organs and lymph glands, and also to the serous membranes. Indeed, in No. 224 tuberculous areas were found in the liver. Koch's assertion that human tuberculosis is different from bovine tuberculosis, and that human tuberculosis can not be communicated to cattle is opposed by this investigator, who found it quite easy to infect cattle with human tuberculosis, although he recognizes that human tubercle bacilli are relatively less virulent for cattle than bovine bacilli. He believes if his animals had been allowed to live longer, and had been subjected to unfavorable conditions such as illness, and so forth, the disease would possibly have been more advanced. In other words, a generalized tuberculosis is not necessary to prove that infection has taken place, as it is well known that bovine tuberculosis can remain local for many years, and at the autopsy only a few small tuberculous areas in the lymph glands are found.

Nocard[22] has been able by the use of collodion sacs containing human or bovine tubercle cultures introduced into the peritoneal cavity of fowls to transform these mammalian tubercle bacilli into cultures which have the morphological and cultural characteristics of avian tubercle bacilli and which possess a virulence for fowls equal to that of the avian tubercle bacillus, but their virulence for guinea pigs was apparently lost.

Svensson[23] attempted to infect healthy calves with tuberculous sputum by means of subcutaneous intratracheal, intraperitoneal, and intrapulmonary injections or through the inhalation of pulverized sputum. About six months after the beginning of the experiment the animals were killed, and the autopsies showed that the serous membranes, as well as the lymph glands in the neighborhood of the inoculations, were tuberculous. In the opinion of the author his experiments show that the human tubercle bacillus possesses relatively slight virulence for cattle.

Dean and Todd[24] made some experiments, the object of which was to ascertain whether the human tubercle bacillus undergoes any marked change in virulence for cattle by passage through the pig, cat, rat, rabbit, or mouse. The authors give an account of their experiments in detail and reach the following general conclusions:

(1) That the hog is capable of contracting a rapidly fatal generalized tuberculosis as the result of infection with the human bacillus.

(2) That the human tubercle bacillus is not markedly increased in virulence for the calf by a single passage through the pig, cat, rat, or rabbit.

(3) That the human tubercle bacillus is by no means innocuous to the calf, as the control animal injected directly with sputum contracted an extensive glandular tuberculosis.

The authors used tuberculous sputum for all of their inoculations instead of pure culture. They noted that sometimes the first animals in a series died earlier than those inoculated later with tissue from the first animal. They explain this by calling attention to the fact that in the case of the second inoculation the tubercle bacillus was present almost always alone, whereas in the sputum inoculations at the beginning of the series many other bacteria were associated with the tubercle bacillus, and the earlier death of the first animals in some cases is to be attributed to the action of the associated organisms rather than to a more exalted virulence of the tubercle bacillus.

De Jong[25] describes, in the latter part of his paper, experiments which he made with a bacillus of human origin, which is very virulent for cattle. The material was obtained from a peasant woman 27 years of age, without hereditary taint, whose occupation brought her in frequent contact with animals. This woman was affected with pulmonary tuberculosis and a guinea pig inoculated with some of her sputum became tuberculous. Cultures from the spleen of this guinea pig served for the two following experiments:

A calf 6 weeks old, weighing 65 kilograms, received in the jugular vein 6 c. c. of an emulsion of culture. The animal had not reacted to tuberculin. Five days after the injection the temperature exceeded 40° C., and it remained elevated until death, which took place at the end of nineteen days. The weight had fallen to 53 kilograms. The autopsy showed extensive tuberculous lesions of the lungs, liver, spleen, kidneys, and lymphatic glands. On the pleura were found alterations characteristic of the early stages of pearl disease.

A goat which was likewise perfectly healthy, weighing 17 kilograms, was inoculated in the jugular vein with 5 c. c. of the same emulsion. This animal died in twelve days of a generalized tuberculosis.

Two other inoculation experiments with this same bacillus are also reported. One was an intrapulmonary injection of a calf and was followed by a generalized tuberculosis. The other was a subcutaneous inoculation of a calf with the same culture, death taking place at the end of fifty-six days. The weight had fallen from 58 kilograms at the beginning of the experiment to 53 kilograms at the time of death. The autopsy revealed very severe generalized tuberculosis, involving the lungs, liver, spleen, kidneys, and lymphatic glands.

It can not be denied that the above case of human tuberculosis is an instance of infection by the bovine bacillus, or by a human bacillus which possessed equal virulence with the bovine bacillus for cattle. Either one of these hypotheses contradicts the theory put forth by Koch.

Wolff[26] mentions a case of primary human intestinal tuberculosis, the virus from which produced generalized tuberculosis in a calf. This case was previously reported in the Deutsche Med. Wochenschrift, 1902, No. 32, where all the details are given of the care which was taken in the inoculation of the virus into a tuberculosis-free calf (tested with tuberculin); also of the clinical course of the disease which followed the subcutaneous injection into the neck, the fact that the animal twice gave an exquisite tuberculin reaction at four and eleven weeks after injection, and, finally, the autopsy upon the calf and the microscopic examination eighty-three days after the beginning of the experiment. The autopsy showed the greatest alterations at the point of inoculation, and in the inner organs changes that are typical of perlsucht. Briefly, the autopsy record is as follows: At the point of inoculation in the neck were found the oldest lesions. A tumor about the size of a child's head with a central cavity which contained a caseous pus, the cavity surrounded by a thick granulation membrane and sprinkled with calcareous deposits. In the neighborhood of the cavity were several glands the size of a small apple, with numerous caseous and calcareous nodules. In the neighborhood of the point of injection the shoulder glands and the pharyngeal glands were greatly swollen with caseous and calcified nodules. The pleura, pericardium, omentum, and the capsules of the spleen and liver were covered with characteristic tumors which varied in size from a millet seed to lentil, bean, and cherry sized nodules. In addition, tumors were found on the serous membranes and in the lung tissue. In the pulp of the spleen, and in the parenchyma of the liver and kidneys, miliary nodules were visible. The microscopic examination of these nodules frequently showed typical epitheliod-celled tubercles, together with giant and round cells, their structure being very similar to the human tubercle. Tubercle bacilli were found in the nodules of all the organs. Upon section and examination of the microscopic preparations, caseation and calcification of the tuberculous new growths at the point of injection and the neighboring glands were found, while the nodules in all distant organs (pleura, pericardium, lung, omentum, liver, spleen, and kidneys) were still fresh and showed neither caseation nor calcification. This microscopic evidence in connection with the identical macroscopic appearance, namely, the fact that the calcification and caseation were found only at the point of injection and in the neighboring glands, and, further, the clinical symptoms which were observed, indicate with certainty that the tuberculous process began first of all at the point of injection and spread from there, producing a general infection of the organism. Taking everything into consideration, there can be no doubt that here is a case of virulent perlsucht in a calf, which was brought about by the inoculation of material from a case of primary intestinal tuberculosis in man. According to Koch, such an inoculation of a calf with tuberculous material from such a case of primary intestinal tuberculosis will enable us to determine positively whether the infection was of human or bovine origin.

The author concludes his paper with a general discussion of the tuberculosis question, and takes the position that even if all the experimental proof of the identity of human and bovine tuberculosis is faulty, we should still, so long as there is any doubt about the transmissibility of bovine tuberculosis to man, take precautions to prevent possible infection.

Theobald Smith[27], in a recent communication to the Association of

American Physicians, described a culture of tuberculosis, isolated from the mesenteric gland of a child, which corresponded morphologically and culturally quite closely to the bovine bacilli with which it was compared. A steer inoculated intrathoracically with this culture became quite ill and was killed thirty-eight days after the inoculation. The autopsy and subsequent microscopic examination revealed tuberculosis of the lungs, mediastinal lymph glands, the liver, and the spleen. The pathogenic power of the culture appeared to be slightly below that of the ordinary bovine tubercle bacillus, but it undoubtedly possessed the ability to produce a genuine tuberculosis in the inoculated steer. Doctor Smith is of the opinion that this organism, on account of its cultural characters, its morphology, and its pathogenic power, should be considered as a bovine bacillus, and that the child from whom the culture was obtained was probably infected directly from cattle through milk.

Park[28] fed tuberculous sputum to four nonreacting calves and at the same time inoculated them subcutaneously with similar sputum. A fifth calf served as a control. After two and one-half months three of the calves reacted to tuberculin. At the autopsy one of them showed a few enlarged lymph glands, but without tubercles. The others had gained in weight. The tuberculin reaction in these cases Park attributes to the abscess at the seat of inoculation.

Mursajew[29] conducted a number of experiments to determine the relation between human and avian tuberculosis. He isolated a number of avian cultures and inoculated guinea pigs, rabbits, and pigeons with both varieties. He found that pigeons are generally fairly resistent to avian tuberculosis and says that their susceptibility to human tuberculosis is not very great, although their susceptibility to both organisms is influenced considerably by starvation. A passage of human tubercle bacilli through the pigeon's body does not increase their virulence for pigeons. Guinea pigs are quite susceptible to both forms of tuberculosis. There are certain slight differences in the pathological changes produced by the two strains of tubercle bacilli, but they are slight and amount to very little. Of five rabbits the author succeeded in infecting two with avian tuberculosis. He does not believe that the virulence of tubercle bacilli is increased by their passage through the bodies of animals.

Karlinski[30] reports fourteen attempts to communicate tuberculosis to cattle. The human cultures were all derived from sputum. Cattle were inoculated by different methods, some intravenously, some intraperitoneally, some subcutaneously. Certain cattle were inoculated with the human bacillus cultivated directly upon Hesse's medium without passage through the animal body. Others were inoculated with the same cultures which had been passed through several guinea pigs. In certain instances the human virus was transferred from one calf to another in order to determine the effect of such passages upon the virulence of the human bacillus. Certain of the animal cultures were grown upon beef serum which was derived from an animal which had been previously inoculated with human tuberculosis. The author considers that thirteen out of fourteen experiments were successful in communicating human tuberculosis to cattle; also that the virulence of the tubercle bacilli was sensibly increased by passage through guinea pigs, and that human tubercle bacilli which have passed through cattle are markedly increased in virulence for cattle. As a result of these experiments Karlinski states that it is possible to communicate human tuberculosis to the usually resistant Bosnian cattle; that the disease affects primarily the lymph system, but a generalized tuberculosis may be produced by certain means, such as increase of virulence by passage through guinea pigs or cattle, and by intravenous inoculations. In his opinion no distinction should be made between human and bovine tuberculosis.

Fibiger and Jensen[31] quite recently reported experiments with 5 additional human tuberculosis cultures. These cultures were obtained from: (1) A man 92 years old, who died of primary intestinal tuberculosis; (2) a girl 12 years old, who died of a generalized tuberculosis which apparently originated in the intestine; (3) a girl 7 years old, who died of a tuberculosis which had its origin in the intestine; (4) a man 40 years old, who died of an intestinal tuberculosis; (5) a child 3½ years old, who died of a generalized miliary tuberculosis.

Material taken from case No. 1 was apparently completely lacking in virulence for a calf when inoculated subcutaneously. A calf inoculated subcutaneously with material taken from a mesenteric gland of case No. 2 developed a tuberculosis of the shoulder gland and of the inferior glands of the neck. This culture evidently possessed little virulence for cattle. The spleen of a guinea pig which had been inoculated with a portion of a mesenteric gland of case No. 3 caused a generalized tuberculosis in a calf on subcutaneous inoculation. Material from a mesenteric gland from case No. 4 produced generalized miliary tuberculosis in a calf on subcutaneous inoculation. A portion of the liver of a guinea pig which contracted tuberculosis as the result of inoculation with material from case No. 5 was used for the subcutaneous inoculation of a calf in which it produced a localized tuberculosis associated with enlargement, caseation, and calcification of the shoulder and of the neck glands on the side of inoculation.

Among the ten cases quoted by Fibiger and Jensen in their two papers, we find that of the four adults from whom material was obtained one furnished a virus which was capable of causing a generalized tuberculosis in cattle. The other three were either entirely lacking in virulence or of only very weak virulence for cattle. Of the six children, four furnished tubercle bacilli of great virulence and two furnished bacilli of low virulence for cattle. Eight of the cases of human tuberculosis were apparently due to a primary intestinal infection. Out of five cases of primary intestinal tuberculosis in children, four furnished bacilli which were of marked virulence for cattle.

The German Commission, headed by Kossel[32], which was appointed to inquire into the various questions raised by Koch in his London paper, reported the isolation of thirty-nine cultures from various cases of human tuberculosis. Nineteen of these cultures produced no lesions in cattle after subcutaneous inoculation; nine cultures produced very slight tuberculous changes in the prescapular gland adjacent to the point of inoculation; seven cultures caused a more marked affection of the prescapular glands, but the disease did not spread to other portions of the lymphatic system; and the remaining four cultures of human origin caused a generalized tuberculosis in the calves which were inoculated subcutaneously with them. It will be seen, therefore, that four of the human tuberculosis cultures, or slightly more than 10 per cent, produced generalized tuberculosis in cattle, and it is very significant that these four most virulent cultures were all obtained from children, who, it is generally admitted, are in more danger of exposure to bovine tuberculosis through food than adults who consume proportionately very much less milk.

COMBINED FEEDING AND INOCULATION EXPERIMENTS.

De Haan[33], after a few subcutaneous and intravenous inoculations of goats, cattle, monkeys, and a horse, and after some feeding experiments with monkeys, a pure culture of human tuberculosis from sputum being used in all cases, formulates the following conclusions:

> (1) It is possible to bring about in Javanese goats, cattle, and horses, by inoculation with human tubercle bacilli from sputum, certain pathological changes in the tissues which, macroscopically as well as microscopically, agree perfectly with the lesions found in Europe in animals which have been infected in a natural manner. The animals which showed these changes were not in the best of health at the time of inoculation. Perfectly healthy sheep and cattle were able to resist infection with the tuberculous material.
>
> (2) There does not exist in the above-named animals a race immunity from tuberculosis. The absence of this disease among the animals of Java must be ascribed to other causes.
>
> (3) The monkey is quite susceptible to infection with tubercle bacilli of human origin.
>
> (4) The feeding of material containing tubercle bacilli can give rise to a tuberculosis of the lungs and of the bronchial lymph glands, together with—
>
> (a) A tuberculosis of the mesenteric lymph glands without perceptible change in the intestines;
>
> (b) Without any other localization of the tuberculous process.
>
> (5) The lungs are the point of predilection for the development of tuberculosis.

Delépine[34] used a mixture of various tuberculous sputa, because tubercle cultures are apt to lose in great part their virulence and also because tuberculous material from man varies greatly in virulence. Six different specimens of sputa were obtained from the Manchester Consumption Hospital and thoroughly mixed. Four calves, which had not been tested with tuberculin because of the possibility that tuberculin might prevent the disease, were inoculated—one in the lung, one subcutaneously, one intraperitoneally, and one was fed milk containing tuberculous sputum. The following results were reported: Calf No. 1, which received 5 c. c. of sputum mixture in the lung, died on the sixth day and showed on postmortem generalized tuberculosis which could not be referred to the inoculation. Calf No. 2, which received 5 c. c. of the mixture under the skin of the leg, died on the sixth day and showed enlargement of a gland 5 inches from the point of inoculation and no other trace of tuberculosis. Live virulent tubercle bacilli were found in the skin. Calf No. 3 received 50 c. c. of sputum mixture with its food in one day; died on the twenty-sixth day; tuberculosis of the esophageal glands, which contained virulent tubercle bacilli. Calf No. 4 received 5 c. c. of sputum mixture intraperitoneally. Twenty-six days after inoculation did not react to tuberculin; sixty-eight days after inoculation gave typical reaction; killed twenty days later and showed plainly tuberculosis of the peritoneum, spreading also to the pleura and pericardium. In addition, some lymph glands which were in relation with the peritoneum were tuberculous.

In these experiments only two calves lived long enough to give definite results. Both of these calves were affected with tuberculosis—one from feeding and the other from intraperitoneal injection with human tuberculous sputum. It must be admitted that the value of Delépine's work is very seriously affected by the fact that one of his calves died a few days after the beginning of the experiment of a recently developed generalized tuberculosis.

Hamilton and Young[35] for their experiments selected, first, calves varying in age from two days to ten weeks. The stall in which the animals were kept had never been occupied by cattle before. The experimental animals were fed upon milk, some meal, and a small amount of hay. The milk was obtained from cows which had not reacted to tuberculin, and every endeavor was used to avoid infection from the outside.

First experiment: Five calves were fed with milk containing human tuberculous sputum. Four of these animals were killed in from sixty-five to sixty-nine days after the beginning of the feeding and showed tuberculous changes in the retropharyngeal, mediastinal, and neck glands. The tuberculous character of the lesions was demonstrated by the presence of tubercle bacilli and giant cells and by the development of tuberculosis in guinea pigs which were inoculated with tissue. One of the calves died of septicemia thirty-seven days after the beginning of the experiment.

Second experiment: Four calves were inoculated subcutaneously with small fragments of a lymph gland taken from a tuberculous human being. Three of these animals were killed in from sixty-six to eighty-seven days after the inoculation. One calf died twenty-two days after the beginning of the experiment. Of the first three, one remained healthy. The second showed tuberculous changes at the point of inoculation, in the neighboring lymph glands, and in the retropharyngeal and mesenteric lymph glands. In the latter case tubercle bacilli were found. In the fourth calf a number of lymph glands were diseased and isolated tuberculous areas were found in the lung. Another calf was inoculated subcutaneously with a fragment of lung taken from a man who had died of miliary tuberculosis. Eighty-seven days after the inoculation the animal was killed and tuberculous changes were noted at the point of inoculation, together with caseation of many lymph glands, and in addition two thickened areas at the base of the left lung. With the caseous material from the bronchial glands guinea pigs were made tuberculous. Finally, another calf was inoculated subcutaneously with urine containing tubercle bacilli which had been obtained from a man. This animal was killed fifty-five days after inoculation and showed enlargement and caseation of the retropharyngeal, mesenteric, and bronchial lymph glands and, in addition, tuberculous areas in the right lung.

Third experiment: Two calves were inoculated subcutaneously with fragments of tuberculous glands from men. In these cases also, when the animals were killed, more or less tuberculosis was found.

A third animal was inoculated subcutaneously with tuberculous sputum. After one hundred and fifty-five days it showed tuberculous bronchial and mesenteric glands. The point of inoculation seemed to be healthy. Two other calves which had been subjected twice to the tuberculin test and had not reacted inhaled tuberculous sputum and showed, one hundred and forty-five days later, marked tuberculosis of the lungs.

Fourth experiment: In order to ascertain what influence the passage of human tubercle bacilli from animal to animal would have upon the virulence of the microorganism, two calves were inoculated subcutaneously with material taken from another calf which had been previously inoculated with human tuberculous sputum. The experiment resulted in emaciation and loss of appetite of the animals. The autopsy showed generalized tuberculosis. As a result of the above experiments the authors reach the following conclusions:

Although the human tubercle bacillus is apparently not so virulent for calves as the bovine bacillus, yet it can infect cattle. The infection may be brought about by means of tuberculous sputum and tuberculous lymph glands or lung tissue. The disease may be brought about through intravenous or subcutaneous inoculations, through inhalation of pulverized sputum containing tubercle bacilli, or through feeding tuberculous sputum. In the case of feeding, the abdominal lymph glands may become infected without visible lesions of the intestinal walls. The passage of the human tubercle bacillus through calves appears to heighten its virulence enormously. The authors, from the above experiments, are of the opinion that the human tubercle bacillus is identical with the bovine bacillus, although somewhat weaker in virulence.

The literature reviewed in the foregoing pages has been selected because of its special bearing upon the experiments we have made, and this review is not to be considered complete. For a fuller discussion of this subject, especially in its relation to public health, the reader is referred to Salmon's[36] report to the American Public Health Association on "Bovine Tuberculosis and Public Health."

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U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY—Bulletin No. 52, Part III.
D. E. SALMON, D. V. M., Chief of Bureau.

EXPERIMENTS CONCERNING TUBERCULOSIS,

UNDER THE SUPERVISION OF

THE BIOCHEMIC DIVISION.

PART III.

Studies in Immunity from Tuberculosis,

BY

E. C. SCHROEDER, M. D. V.,
Superintendent of Experiment Station, Bureau of Animal Industry,

AND

The Persistence of Tubercle Bacilli in Tissues of Animals after Injection,

BY

E. C. SCHROEDER, M. D. V., AND W. E. COTTON,
Experiment Station, Bureau of Animal Industry.

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1905.

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U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY—Bulletin No. 52. Part III.
D. E. SALMON, D. V. M., Chief of Bureau.

EXPERIMENTS CONCERNING TUBERCULOSIS,

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Experiment Station, Bureau of Animal Industry.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1905.

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., March 3, 1905.

Sir: I have the honor to transmit herewith two articles which comprise the third and final installment of the series entitled "Experiments concerning tuberculosis," undertaken by the Biochemic Division of this Bureau. Parts I and II of the series have already been published.

The first article deals with a number of investigations made with special reference to the production of immunity from tuberculosis. It should here be stated that the late Dr. E. A. de Schweinitz, as chief of the Biochemic Division, was interested in this portion of the work, but as he left no notes or memoranda expressive of his views he can not properly be held responsible for any of the statements herein made.

The second article concerns the power of persistence of certain tubercle bacilli which are of insufficient virulence to cause real tuberculosis, but which remain in the tissues of cattle after subcutaneous or intravenous injection. This subject is apt to be of considerable general importance in view of the reasons mentioned in this part of the work, added to the fact that the observations tended strongly to the conclusion that such bacilli became incorporated with the tissues and remained in them for long periods without losing their infectious character.

I recommend that the articles be published as Part III of Bulletin No. 52 of this Bureau.

Respectfully,
D. E. Salmon,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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PART III.—STUDIES IN IMMUNITY FROM TUBERCULOSIS; AND THE PERSISTENCE OF TUBERCLE BACILLI IN ANIMALS AFTER INJECTION.

STUDIES IN IMMUNITY FROM TUBERCULOSIS.

By E. C. SCHROEDER, M. D. V.,
Superintendent of Experiment Station, Bureau of Animal Industry.

In the Medical News of December 8, 1894, the late Dr. E. A. de Schweinitz called attention to a bacillus of tuberculosis, isolated from human tuberculous tissue, which was of special interest because its original pathogenic virulence for small experiment animals had disappeared during its cultivation in the laboratory of the Biochemic Division on artificial media, and because animals injected with it were perceived—on subsequent injection with more virulent tuberculous material—to have acquired a certain degree of immunity to tuberculosis. A second communication on the bacillus was made in Bureau of Animal Industry Bulletin No. 13, issued September 19, 1896, and since then additional experiments with reference to its immunizing qualities have been completed. The last-named experiments, in the light of the investigations of Behring, McFadyean, Pearson, Gilliland, and others, on the question of immunization to tuberculosis, must be of special interest at the present time, and they, together with some other work in which Doctor de Schweinitz was interested, form the subject of the present article.

EXPERIMENTS WITH ATTENUATED TUBERCLE CULTURE.

In the year 1896 two calves received injections of attenuated tubercle culture,[a] and were later inoculated with virulent bovine tuberculous tissue, and then treated with additional copious injections of attenuated tubercle culture. They were afterwards killed and found to be affected with tuberculosis. Their records follow:

Record of calf No. 27.—About 6 months old, injected subcutaneously with attenuated tubercle culture as follows: April 15, 1896, 10 c. c.; April 16, 1896, 25 c. c., and April 29, 1896, 50 c. c. The culture contained much flocculent growth. On May 8, 1896, inoculated subcutaneously with tuberculous tissue from a guinea pig which had succumbed to bovine tuberculosis. On June 26, 1896, the calf was tested with tuberculin and failed to react. From June 30, 1896, to August 22, 1896, at regular intervals, six additional subcutaneous injections of attenuated tubercle culture were made, in doses from 100 to 500 c. c., a total quantity of 1,750 c. c. On April 17, 1897, the calf was killed and examined postmortem. The autopsy revealed generalized tuberculosis. The superficial lymph glands near the seat of inoculation with the virulent tuberculous tissue were enormously enlarged, completely necrotic, and presented several areas of softening. It was clear that the disease began at this point and spread from it to the internal organs.

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[a] The term "attenuated tubercle culture," wherever it is used in this article, refers to the culture discussed in the Medical News of 1894 and Bureau of Animal Industry Bulletin No. 13, 1896.

Record of calf No. 42.—About 5 months old. From April 15, 1895, to October 15, 1896, received eleven subcutaneous injections of attenuated tubercle culture, in doses from 10 to 500 c. c. The culture contained much flocculent growth. On October 23, 1896, an intra-abdominal injection of tuberculous tissue from a guinea pig which had succumbed to bovine tuberculosis was made. The calf was killed on April 27, 1897. The autopsy revealed a few subpleural tubercles in the lung, a sprinkling of small tubercles over the pulmonary surface of the diaphragm, and over the omentum, and a few small tubercles in the spleen. The entire absence of disease in the lymph glands is notable.

The injections of attenuated tubercle culture apparently did not give much protection, and the work along this line for the production of immunity was temporarily dropped, with the exception that two cows, which had received injections of large quantities of attenuated tubercle culture in an effort made to cause them to yield an antituberculous blood serum, were tested, relative to immunity, with inoculations of bovine tuberculous material. A condensed record of the two cows—Nos. 217 and 297—one of which resisted the inoculation of virulent tuberculosis, follows:

Record of cow No. 217.—About six years old, in excellent condition, tested with tuberculin without reaction. July 26, 1894, injected into right jugular vein 16 c. c. of attenuated tubercle culture. November 19, 1894, tested with tuberculin without reaction. November 28, 1894, injected intraabdominally with 6 c. c. of attenuated tubercle culture. January 25, 1895, and March 5, 1895, respectively, injected into thorax 6 and 16 c. c. of attenuated tubercle culture. The first of the above injections was followed by the development of a small abscess at the point where the syringe needle punctured the skin over the jugular vein. The abscess healed slowly. The second injection caused a moderate amount of fever, which disappeared in two days, and the third and fourth injections were unattended by visible effects. From April 27, 1895, to February 13, 1896, nine subcutaneous injections of attenuated tubercle culture were made, in doses varying from 36 to 200 c. c. Almost every injection in this series was followed by the formation of an abscess. The abscesses broke in ten to twelve days, discharged a thick, creamy pus which contained innumerable tubercle bacilli, and healed slowly under antiseptic treatment. The magnitude of the abscesses was proportional to the amount of material injected, and the larger abscesses affected the general health of the cow in a noticeable but not serious degree. On August 22, 1895, the cow aborted in the fifth month of pregnancy. From March 30, 1896, to July 7, 1897, thirty subcutaneous injections of attenuated tubercle culture were made. The injections differed from the preceding nine in that the cultures used were filtered through paper. This was done because the abscesses in the earlier injections were attributable to the large flakes of tubercle germs held in suspension in the cultures. The doses of filtered culture varied from 250 c. c. to 2,000 c. c., and the total amount injected was 19,700 c. c. This last series of injections was accompanied by the occasional formation of a small abscess at the seat of injection, which healed slowly under treatment and did not affect the general health of the cow. On November 27, 1896, a healthy calf was produced, which was tested several times with tuberculin without reaction, and was free from tuberculosis and in excellent condition in the year 1901.

Blood was drawn from the cow on the following dates: November 21, 1895, 3,000 c. c.;. January 14, 1896, 1,500 c. c.; July 31, 1896, 3,000 c. c., and July 31, 1897, 1,800 c. c. The antituberculous potency of the blood serum was tested by the late Dr. E. A. de Schweinitz, whose notes on the subject are not now accessible. He told me, however, shortly after the tests were made, that the serum had little or no value. February 22, 1898, the cow was given an intrathoracic injection of 10 c. c. of an emulsion made by triturating with sterile water a tuberculous gland from a cow affected with generalized, natural tuberculosis. A number of cover glasses smeared with the emulsion were stained and examined microscopically, and every one showed the presence of tubercle germs. On September 20, 1898, about seven months after the last injection, the cow was killed and examined postmortem. She was fat and healthy, and no lesions of diseases were found.

Record of cow No. 297.—Born at the Experiment Station on October 19, 1894, daughter of tuberculous cow No. 285. Tested with tuberculin without reaction when one month old. November 24, 1894, received an intraabdominal injection of 6 c. c. of attenuated tubercle culture; January 25, 1895, an intrathoracic injection of 16 c. c. of attenuated tubercle culture; and on April 7, June 1, and July 25, 1895, respectively, 26, 75, and 100 c. c. of attenuated tubercle culture were injected subcutaneously. On August 9, 1895, an intrathoracic injection was made of material consisting of the growth scraped from the surface of a blood-serum bovine tubercle culture, suspended in 6 c. c. of sterile beef broth. From December 2, 1895, to February 13, 1896, five subcutaneous injections of attenuated tubercle culture were made, in doses ranging from 50 to 150 c. c., a total of 400 c. c. From March 20, 1896, to July 17, 1897, thirty subcutaneous injections of attenuated tubercle culture filtered through paper were made, in doses ranging from 85 to 1,750 c. c., or a total of 13,325 c. c. On February 22, 1898, received an injection into the thorax consisting of 10 c. c. of an emulsion made by triturating with sterile water a tuberculous gland from a cow affected with generalized natural tuberculosis.

The cow was in poor condition throughout the entire experiment. The injections of unfiltered tubercle culture made between August 7, 1895, and February 13, 1896, invariably produced abscesses, which broke in ten days to two weeks, discharged a thin, necrotic material which contained innumerable tubercle bacilli, and healed slowly under antiseptic treatment. The abscess which followed the injection of February 13, 1896, was especially large, and it was feared at one time that it would cause the death of the cow. The injections of filtered culture occasionally produced small abscesses, which healed slowly under treatment after discharging necrotic material of the kind described above. On December 17, 1896, a very weak, small calf was born, which died January 19, 1897. Autopsy examination of the calf showed no lesions of tuberculosis. On September 19, 1898, a second calf was born dead, somewhat prematurely. This calf, like the other, showed no lesions of tuberculosis on postmortem examination.

The results produced by the injection of bovine tuberculous material on August 9, 1895, were not accurately determined. Blood was drawn from the cow several times and tested by the late Dr. E. A. de Schweinitz, who told me shortly after the tests were completed that the serum had no antituberculous value. On September 20, 1898, the cow was killed and examined postmortem. Her general condition was fairly good. Large tuberculous masses were found on the pulmonary and costal pleura and several large, broken-down tubercular areas in the lung tissue. Right hind quarter of udder was sprinkled with numerous small tubercular nodules. No other lesions observed. As in the case of calf No. 42, the lymph glands were all healthy, those associated with the extensively diseased lung and udder included.

It is somewhat remarkable, especially in the light of results obtained by more recent investigations, that the only animal of the four treated with attenuated tubercle culture which remained unaffected after inoculation with virulent tubercular material received, among others, one intravenous injection.

EXPERIMENTS WITH BLOOD SERUM.

In conjunction with the attempt made in the earlier experiments to immunize cattle to tuberculosis by injections of attenuated tubercle culture, an effort was made to gain immunity through the injection of blood serum from a tuberculous cow which had received copious injections of tuberculin. The animals injected with blood serum were calves Nos. 36 and 41. The animal which yielded the serum was tuberculous cow No. 285, and her record will be presented in full, followed by that of the calves.

Record of cow No. 285.—Obtained from a dairy herd in Virginia in the summer of 1894 after she had given a typical reaction to tuberculin. She was thin but not visibly affected with tuberculosis. Her age was about 7 years. On October 19, 1894, she gave birth to an apparently healthy calf (cow No. 297). From August 22, 1895, to October 2, 1896, she received 49 subcutaneous injections of a 12½ per cent solution of tuberculin, in doses ranging from 25 to 1,500 c. c. The total amount of tuberculin solution injected in this series was 8,097 c. c. From October 16, 1896, to November 13, 1896, she received 3 subcutaneous injections of a 33⅓ per cent solution of tuberculin, amounting to 4,320 c. c. From February 8, 1897, to July 17, 1897, she received 16 subcutaneous injections of a 12½ per cent solution of tuberculin, in doses ranging from 250 to 1,000 c. c., a total amount in this last series of 7,000 c. c. At first the cow seemed to show some improvement under the treatment, then her condition was stationary for several months, and then slowly declined. An interruption in the injections of two months and twenty-six days occurred between November, 1896, and February, 1897. During this time her condition declined very rapidly. When the injections were resumed, her condition at first improved and then became stationary. When the injections were finally stopped, the condition of the cow declined very rapidly, and on October 8, 1897, when she was killed, less than three months after the last injection was made, she had been down and unable to regain her feet for several days. The total amount of tuberculin injected was 15,097 c. c. of 12½ per cent solution and 4,320 c. c. of the 33⅓ per cent solution, or the equivalent of 3,327⅛ c. c. of the concentrated extract from tubercle cultures.

Autopsy record: Extremely emaciated. The costal and pulmonary pleuræ are greatly thickened and covered with tubercles of all sizes and in all stages of development and degeneration. Numerous tubercles are present in the lung tissue. The mediastinal and mesenteric glands are enormously enlarged and completely tuberculous. Several of the superficial lymph glands, in various portions of the body, are converted into tuberculous abscesses containing soft, cheesy, necrotic material.

The injections of tuberculin, copious as they were, failed to heal the tuberculosis with which the animal was affected, but it is probable that the disease was somewhat retarded by them. This conclusion is justified by the rapid decline in general condition when the injections were suspended between November, 1896, and February, 1897, the improvement when they were resumed, and the rapid progress to a fatal termination when they were entirely stopped.

Blood was drawn from the cow at regular intervals five times, and in addition to the injection of the two calves it was tested on a number of small experiment animals and was found to have no antitubercular value.

Record of calf No. 36.—About 6 months old. Received 3 subcutaneous injections of blood serum from cow No. 285, as follows: April 15, 1896, 50 c. c.; April 16, 1896, 75 c. c., and April 29, 1896, 75 c. c. On May 8, 1896, received a subcutaneous injection of an emulsion made by triturating with sterile water a portion of a tuberculous gland from a cow affected with generalized, natural tuberculosis. The calf died on August 26, 1896. On autopsy no lesions of tuberculosis were found except at the seat of the injection made in May. The cause of death was inflammation of the bowels.

Record of calf No. 41.—About 5 months old. Received 3 subcutaneous injections of blood serum from cow No. 285, as follows: April 15, 1896, 50 c. c.; April 16, 1896, 75 c. c., and April 29, 1896, 75 c. c. On October 23, 1896, received an intraabdominal injection consisting of an emulsion made by triturating with sterile water a portion of a tuberculous gland from a cow affected with generalized, natural tuberculosis. The calf was killed on April 27, 1897, and on autopsy one of the lymph glands at the root of the tongue was found to be enormously enlarged and converted into a broken-down, tuberculous cyst. Numerous minute tubercles were found in the left principal lobe of the lung near its apex. There were no other lesions.

It is curious that the disease in this case, which probably resulted from the intraabdominal injection of tuberculous material, was confined to the throat and thorax. Not much information can be obtained from the two calves regarding the antituberculous value of the blood serum from cow No. 285, because calf No. 36 died too early after inoculation, and the interval of time which elapsed between the serum injections and the inoculation of calf No. 41 was probably too long for the persistence of an immunity acquired through the injection of an antitoxin. The guinea-pig tests made along the same line gave results which offered no encouragement to sacrifice additional cattle in the work.

EXPERIMENTS WITH WEAK VIRULENT CULTURES.

It having been definitely established that many cultures of tubercle bacilli of decided virulence for guinea pigs are innocuous for cattle, this fact suggested a possibility of gaining additional light on the question of immunization. Accordingly, in the years 1901 and 1902 several bovine animals were injected with tubercle bacilli, supposedly not infectious for them, but of much greater virulence than our attenuated tubercle culture. Four cattle were thus treated, two[a] were injected subcutaneously in September, 1901, with virulent tubercle culture of human origin, and two intravenously with similar cultures in July, 1902. The four animals were afterwards exposed to tuberculosis with checks, or untreated animals, and the results show that they undoubtedly gained some degree of immunity. The records of the animals follow:

Subcutaneous injections.—Heifer No. 213, born at the experiment station November 24, 1900, tested with tuberculin without reaction September 4, 1901. Condition very good. On September 24, 1901, received a subcutaneous injection back of left shoulder of 10 c. c. of human tubercle culture strongly virulent for guinea pigs. The culture contained much flocculent growth. The injection was followed by the development of a tumor, 3 by 6 by 18 cm. in size, which was at first hard, feverish, and sensitive, but gradually softened into a nonsensitive, fluctuating abscess. On December 3, 1901, a tuberculin test was made and no reaction obtained. On February 4, 1902, a fluctuating, nonsensitive tumor, 5 by 7 by 10 cm., was present at seat of injection. This was punctured and a small amount of broken-down necrotic material withdrawn and examined under the microscope and found to contain innumerable tubercle bacilli. Four guinea pigs inoculated with this material became affected with generalized tuberculosis. The heifer was again tested with tuberculin on April 5, 1902, and failed to react. The tumor at the seat of injection after it was opened gradually diminished in size, and by the end of August had shrunken to about 3 cm. diameter. On September 19, 1902, a subcutaneous injection back of right shoulder of 10 c. c. of bovine tubercle culture was made. This was followed by the formation of a tumor, first at the seat of injection, and then a second tumor in the region of the prescapular gland, these tumors remained until the animal was killed. On October 24, 1903, a tuberculin test was without reaction. On November 2, 1903, the heifer was killed and examined postmortem. The autopsy revealed a small abscess at the seat of human tubercle injection, which contained a thick, cream-colored, necrotic material. At the seat of the bovine tubercle injection a similar but much larger abscess was found, and the lymph gland near this was greatly enlarged and contained two foci of tuberculosis, each about 10 mm. diameter. No other lesions of disease.

Steer No. 197, born at the experiment station May 27, 1900. The record of this animal is in all practical respects similar to that of the preceding animal—heifer No. 213—with the exception that on autopsy six small foci of tuberculosis in the place of two were found in the lymph gland nearest the seat of injection with bovine tubercle culture. The steer was killed November 3, 1903.

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[a] Another—calf No. 221, mentioned on page 57 of Part II of this bulletin—was, along with a check, exposed to infection under conditions which had caused other animals to contract tuberculosis, but as the check animal remained unaffected the experiment proved nothing on the question of immunization.

A check, bull No. 208, was inoculated with bovine tubercle culture on the same date with the two foregoing animals. With respect to age, size, and condition this animal was as much like the other two as possible. It was kept under observation at the experiment station fully a year before it was used in the experiment and tested several times with tuberculin without reaction. A tuberculin test made a few days before it was killed gave a high, characteristic reaction of the presence of tuberculosis. On autopsy it was found to be affected with completely generalized tuberculosis. The bull was killed November 3, 1903.

It seems reasonable to conclude from the foregoing that heifer No. 213 and steer No. 197 had acquired a considerable degree of protection.

Intravenous injections.—Calf No. 259, born at the experiment station on February 12, 1902. Tested with tuberculin without reaction on May 1, 1902. On July 19, 1902, received an intravenous injection of 10 c. c. of human tubercle culture of the same kind as that which was used for the subcutaneous injection of cattle Nos. 213 and 197. The injection was followed in a few days by accelerated respiration, cough, loss of appetite, and great depression. The symptoms increased in severity until the end of July and persisted until the end of August, when the calf had become very thin and was spiritless, had a frequent moist cough, and lacked appetite. Early in September some improvement was apparent, but the animal did not regain its original condition until January, 1903. On January 24, 1903, a subcutaneous injection of 5 c. c. of virulent bovine tubercle culture was made in front of the right shoulder. The injection was followed by the formation of a firm tumor, which remained until death. A tuberculin test made on October 24, 1903, gave a fairly characteristic reaction. The calf was killed on November 3, 1903, and examined post-mortem. At the seat of the bovine tubercle-culture injection a small abscess was found, which contained a thick, creamy pus, and the superficial lymph gland near this region was greatly enlarged and contained several softened foci of tuberculosis. The lung was sprinkled with innumerable minute foci of tuberculosis from 1 to 5 mm. in diameter. No disease of the mediastinal or other thoracic lymph glands was found. The lung lesions were in all practical respects similar to the lesions found in the lungs of other cattle at the experiment station which had received intravenous injections of human tubercle culture and no subsequent injections of bovine or more virulent tubercle culture; that is, a miliary tuberculosis of the lung without disease of the thoracic lymph glands. This fact, together with the symptoms that followed the earlier or intravenous injection of human tubercle culture, leaves no doubt that the earlier injection was the cause of the lung lesions found in the calf and that the later injection of bovine tubercle culture produced no disease aside from the lesions at the seat of the injection and in the adjacent lymph gland.

A check animal, heifer No. 292, was injected with bovine tubercle culture on the same date on which calf No. 259 was injected. The check was in all respects as nearly as possible like No. 259, with the exception that it had received no form of treatment to protect it against tuberculosis. It was kept at the experiment station several months, and was tested with tuberculin without reaction before it was used in the experiment. On autopsy it was found to be affected with generalized tuberculosis. The heifer was killed November 13, 1903.

The conclusion to be drawn from the records of the last two animals is the same as that from the three preceding them. It can not be said that the intravenous injection conferred a higher degree of resistance to tuberculosis than the subcutaneous injections of the same culture.

Cow No. 211, about 2 years old; tested with tuberculin without reaction. July 19, 1902, 10 c. c. of moderately virulent human tubercle culture injected into right jugular vein. July 25, 1902, gave birth to a healthy calf. August 6, 1902, the above injection repeated. August 9 to 19, 1902, suffered from accelerated respiration and loss of appetite. August 19, 1902, has grown quite thin, but seems to be in good general health. Her poor condition is probably due to the milk secreted for her calf, which is in excellent health and growing vigorously. August 20, 1902, a third intravenous injection of 10 c. c. of moderately virulent human tubercle culture was made; on September 20, 1902, a fourth, and on October 7, 1902, a fifth injection. Each of the five injections was followed by an elevation of temperature of from 2° to 4° F., which lasted from three to seven days, and by more or less acceleration in respiration. December 12, 1902, the calf was weaned. January 27, 1903, tested with tuberculin without reaction, after which she was exposed in a stable which contained, among other cattle, three cows affected with advanced tuberculosis. In order to make the exposure severe, her stall was changed daily, so that she practically occupied a stall each day throughout the exposure which was occupied on the previous day by a tuberculous cow. Her general condition was thin and unthrifty. She was giving about a gallon of milk daily. April 3, 1903, is thin, but seems to be in perfect health. June 10, 1903, is improving in condition; still producing milk. June 30, 1903, tested with tuberculin without reaction. August 6, 1903, killed and examined postmortem. The entire lung is sprinkled with minute white nodules about 1 mm. in diameter. The appearance is similar to that observed in other animals on several occasions at the experiment station resulting from the intravenous injection of human tubercle cultures. There are thousands of little nodules present, and a few as much as 2 mm. in diameter. The mediastinal glands contain a number of circular red patches, but no tubercular lesions. The costal and pulmonary pleuræ show considerable thickening and roughening. The liver contains a number of necrotic areas of parasitic origin. Two guinea pigs were inoculated with fragments from the red circular patches in the mediastinal glands. These guinea pigs were killed two and one-half months later and were found to be in excellent condition and free from all lesions of disease. Two guinea pigs were inoculated with minute nodules from the lung. One died two months and the other was killed two and one-half months after inoculation, and on autopsy both were found to be affected with generalized tuberculosis.

The lesions observed in the cow are undoubtedly the result of the intravenous injections received previous to the exposure. This conclusion is drawn from the fact that it is not probable that a simple stable exposure caused thousands of independent centers of disease, all of approximately the same age, to develop in the lung, without a single tuberculous lesion in any other portion of the body. It is very likely that the germs introduced into the jugular vein passed along the circulation to the right chambers of the heart, and from there to the lung, where they were entirely filtered out of the blood, and caused a tuberculous disease. This disease, it is fair to assume, was of a temporary character, and would have ended in complete recovery if the cow had not been killed. Six other cattle[a] exposed in the same manner as cow No. 211, became affected with tuberculosis which was undoubtedly due to the exposure.

SUMMARY OF THE IMMUNITY EXPERIMENTS.

The conclusions which may be drawn from the foregoing work must be accepted with the clear understanding that additional investigations may modify them very materially.

In the attenuated-culture series of experiments it will be noted that calves Nos. 27 and 42 and cows Nos. 217 and 297 were injected with large amounts of attenuated tubercle culture and were afterwards tested relative to immunity with inoculations of bovine tuberculous tissue. Three of the animals contracted tuberculosis and one remained unaffected.

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[a] See Twentieth Annual Report of the Bureau of Animal Industry, 1903, pages 61 to 68, for further details regarding character of exposure of cow No. 211.

In the experiment with cultures virulent for guinea pigs but innocuous for cattle we find that two animals, Nos. 213 and 197, received each a single injection of virulent human tubercle culture, and subsequently, with a third untreated animal, No. 208, each an injection of virulent bovine tubercle culture. Nos. 213 and 197 became affected with a superficial tuberculosis, strictly localized at or near the seat of injection, while No. 208, untreated, became affected with generalized tuberculosis.

One animal, calf No. 259, received a single intravenous injection of virulent human tubercle culture, and later, together with a second animal, untreated—No. 292—a subcutaneous injection of virulent bovine tubercle culture. The lesions found in calf No. 259 were a probably healing miliary tuberculosis of the lung, induced by the earlier injection, and a superficial tuberculosis, strictly localized at or near the seat of the later injection. The check animal—No. 292—became affected with a generalized tuberculosis.

One animal, cow No. 211, received five injections of virulent human tubercle culture, and was afterwards exposed with six untreated cattle to several cows affected with advanced tuberculosis. The lesions found in cow No. 211 were a probably healing miliary tuberculosis of the lung, attributable to the intravenous injections she had received. The lesions found in the six untreated and similarly exposed cattle were of a kind to leave no doubt that they were the direct result of the exposure.

DISCUSSION OF THE EXPERIMENTS.

A general comparison of the cattle which received injections of tubercle culture to protect them against tuberculosis shows that numerous injections of attenuated culture, both before and after inoculation with virulent tuberculous material, did not afford as much protection as a single injection, subcutaneous or intravenous, of a more virulent tubercle culture. Hence, it seems, the amount of protection an animal can derive from the injection of tubercle cultures which are too weak to cause it to become affected with a progressive tuberculosis is directly proportioned to the virulence of the culture.

The presence of only superficial, localized tuberculosis in cattle Nos. 213, 197, and 259, and a generalized tuberculosis in Nos. 208 and 292, is strong evidence for the conclusion that the injection of a virulent tubercle culture, the virulence of which is insufficient to cause a progressive tuberculosis in the species of animal injected, affords a high degree of immunity to subsequent infection with tuberculosis.

A comparison of the two animals that received subcutaneous injections with the two that received intravenous injections of virulent human tubercle culture seems to indicate that the subcutaneous injections were as effective in giving protection as the intravenous.

Regarding the blood-serum experiments carried on with cow No. 285 and calves Nos. 36 and 41, and the value of the blood serum of cow No. 217, which received enormous quantities of attenuated tubercle culture, these require no further consideration, as the statement has already been made that the blood serum possessed no satisfactory, if any, antituberculous property.

The tuberculous material with which the several cattle were injected represents four degrees of virulence: (1) The attenuated culture, innocuous for guinea pigs; (2) a human tubercle culture, highly virulent for guinea pigs; (3) fresh tuberculous tissue; and (4) a bovine tubercle culture of sufficient virulence to produce generalized tuberculosis in unprotected cattle. The cattle injected with tuberculous tissue and tubercle culture represent practically three degrees of susceptibility to tuberculosis: (1) The untreated cattle; (2) those which received attenuated culture, and (3) those which received protective injections of highly virulent human tubercle culture.

Taking the degrees of virulence on the one hand and the degrees of susceptibility on the other hand, it is interesting to know that the lesions produced by the various subcutaneous injections when compared with each other were found to be of the same general character. This fact seems to imply that the tubercle bacilli in the different kinds of infectious material used, apart from the wide range in virulence they represent, are identical in their pathological significance.

Another fact, which will be treated at greater length in a separate article, merits a reference here. It is the development of lesions at the seats of subcutaneous injections with cultures of tubercle bacilli. The injections made in this series of experiments almost invariably produced local lesions, the only exceptions being when filtered attenuated cultures were used. When material from these lesions was examined and tested, even a long time after the injections were made, it was found to contain numerous live tubercle bacilli. This is a great objection to the use of live tubercle culture injections for producing immunity to tuberculosis in animals, and it is not overcome by substituting intravenous for subcutaneous injections. The intravenous injections affect the animals much more severely, and live tubercle bacilli are found in the numerous small tubercles which develop in the lung tissue.

AN EXPERIMENT TO OBTAIN BLOOD SERUM.

The record of one additional animal may be of interest in connection with the general subject of the injection of attenuated human tubercle culture. The animal is a donkey, and was injected first with tuberculin, then with attenuated tubercle culture of the kind described in the beginning of this article, and finally with attenuated tubercle culture mixed with sterilized cultures of streptococcus. The injections were all made into the subcutaneous tissues. The object for making them was to obtain an animal which would yield an antitoxic blood serum for the treatment of tuberculosis and the frequent attendant lesions of tuberculosis supposed to be due to streptococci. The record of the donkey follows:

Donkey No. 5, female, about 3 years old; in excellent condition. From September 15, 1897, to January 25, 1898, received eleven injections of a 12½ per cent solution of tuberculin, in doses ranging from 5 c. c. to 70 c. c.; the total amount injected was 405 c. c. An injection made December 15 was followed by the formation of a small local abscess which healed rapidly without treatment. With this exception the injections were unattended by either local or constitutional reactions. From February 4, 1898, to April 7, 1898, received eleven injections of attenuated tubercle culture, in doses which were gradually increased from 10 to 125 c. c.; the total amount of culture received was 715 c. c. The injections were occasionally followed by a more or less edematous condition of the subcutaneous structures into which the cultures were introduced, but did not otherwise visibly affect the animal. From April 18, 1898, to June 22, 1899, thirty-six injections of a mixture of attenuated tubercle culture and sterilized streptococcus culture were made. The amount of material injected at any one time was regulated by the general condition of the donkey; the largest single dose of tubercle culture was 150 c. c. The amount of streptococcus culture varied from a minimum of one-half c. c. to a maximum of 50 c. c. at a single injection. The total amount of tubercle culture injected in the last series was 3,902 c. c., and the total amount of streptococcus culture was 586½ c. c. The first twelve injections of the mixed cultures were unattended by a reaction; but every one of the remaining twenty-four injections was followed by a local reaction and a marked disturbance of the donkey's health. As a rule, each successive injection caused a greater local and general effect than the one preceding it. Locally the condition was an edema, beginning at the seat of injection and frequently extending over a whole quarter of the body. This was followed by an abscess at the seat of injection, which in time broke open and discharged a sanguino-purulent fluid, after which its walls sloughed away and left a deep ulcer, lined with necrotic material of a decidedly tuberculous character. These abscesses and ulcers were a great drain on the animal, and healed very slowly under strong antiseptic treatment. The constitutional symptoms were loss of appetite, depression, loss of weight, and moderate fever. The donkey died on July 13, 1899, twenty-three days after the last injection of mixed cultures was made.

Autopsy of donkey No. 5.—General condition poor. Near the seats of several injections, over which the skin has healed perfectly, are groups of small tubercles in the muscular tissue, some of which have softened centers. Back of the right shoulder, near the seat of an injection made on October 25, 1898, over eight months before the donkey died, is a chain of small cheesy tubercles, which extends through the intercostal muscles and ends in a group of tubercles, which cover an area of 6 to 7 cm. in diameter, on the costal pleura. The pleura over the right principal lobe of the lung, which rests against the group of tubercles on the costal pleura, is covered with similar small tubercles, and the underlying lung tissue, to a depth of 3 to 4 cm., contains many small tubercles. The posterior mediastinal glands are converted into large cheesy tumors, in which many small softened areas are present. Liver enlarged, very firm; connective tissue much increased in amount. Kidneys pale, probably some degeneration of the parenchyma. Six guinea pigs were injected with cheesy material from the mediastinal glands. Of these, three were killed on August 11, and on autopsy were found to be affected with generalized tuberculosis. The remaining three guinea pigs died, respectively, August 11, August 16, and August 31, as the result of generalized tuberculosis. Other guinea pigs, injected at different times direct with the attenuated tubercle culture used for the donkey, did not become affected with tuberculosis.

The object of the experiment, as already stated, was the production of an antituberculous serum. The result was that the donkey became affected with tuberculosis and that there was an apparent increase in the virulence of the tubercle bacilli for guinea pigs. It is to be regretted that, owing to the lack of time and facilities, the increase in the virulence of the bacilli could receive no further tests.

Parallel with donkey No. 5, two horses and a mule received injections of attenuated tubercle culture, but no streptococcus culture. These animals were carefully examined after death and no lesions of tuberculosis found.

The serum from the donkey, horses, and mule was tested in various ways by the late Dr. E. A. de Schweinitz, who long believed that it possessed considerable antituberculous value. What his final conclusions were it is now impossible to say, as his notes are not accessible.

The disease which developed in the donkey suggests the possibility of an increase in the virulence of tubercle bacilli in the tissues of animals in the presence of some other germs or toxins produced by germs. If this is the case (its possibility only is referred to here), the indestructible character of tubercle bacilli once they are lodged in the tissues becomes even more significant, and the view expressed from time to time by some investigators that tubercle germs may live in the tissues in a dormant state for a long period of time and suddenly, for some unknown reason, become active, multiply, and cause progressive tuberculosis may closely approach the truth.

THE PERSISTENCE OF TUBERCLE BACILLI IN THE TISSUES OF ANIMALS AFTER INJECTION.

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GENERAL REMARKS AND RECORDS OF ANIMALS.

Tubercle bacilli differ in pathogenic virulence from innocuousness for the most susceptible species of domestic animals to infectiousness for the most resistant: From the attenuated germ which has been under cultivation in the laboratories of the Bureau of Animal Industry for some years, and which fails to produce disease on subcutaneous injection in guinea pigs, to bacilli, isolated from human and bovine tuberculous lesions, which invariably cause generalized tuberculosis in cattle on subcutaneous injection. This difference in virulence has played an important part in the investigations which have been made to discover a means of immunization against tuberculosis, and advantage is taken of it in a method which is to some extent practiced to protect cattle from tuberculosis. The method is, in brief, the introduction of live tubercle germs of low virulence into the bodies of the cattle. The work of the Bureau of Animal Industry, and of Behring, Pearson, and others, has effectually demonstrated that when this is done a high degree of immunity to subsequent exposure to more virulent infection is gained. There are two circumstances, however, which make the practice of the above method questionable. One is the long persistence of the tubercle bacilli in the tissues of the animals, irrespective of their degree of virulence; the other, the relative susceptibility of man to tuberculosis.

If man is more susceptible to tuberculosis than cattle, the method of immunization is certainly not justifiable, because, owing to the long persistence of infectious material in the tissues of immunized cattle, they are an additional source of danger to him. That man is more susceptible may be concluded from the fact that tubercle bacilli isolated from human lesions are rarely infectious for as many species of animals as tubercle bacilli obtained from bovine sources. And even if this conclusion is not altogether warranted, it is much safer to accept it in the light of our present knowledge than to credit human beings with a higher degree of resistance to tuberculosis than they actually possess, and thus increase their chances to contract the disease.

It has been asserted on good authority, although we do not agree with the view, that the tubercle bacilli which affect man and those which affect cattle are different in kind. If this view is preferred to a simple difference in virulence it need only be remembered that the bacilli which are used for purposes of immunization, of low virulence for cattle, are usually derived from human sources, and that in this case the bodies of cattle are temporarily, for an indefinite period of time, made repositories for human tubercle bacilli in order to protect them against bovine tuberculosis. In this article we have collected the various observations we have been able to make on the persistence of live tubercle bacilli in the tissues of cattle. The records of the animals follow:

Record of heifer No. 216.—On August 24, 1901, injected with a mixture of 10 c. c. tubercle culture and 5 c. c. of streptococcus culture. The tubercle culture was isolated from human tuberculous tissue and possessed a high degree of virulence for guinea pigs. The injection was followed by the formation of a local tumor, which was at first hard and sensitive and afterwards was converted into a fluctuating, nonsensitive abscess. The heifer was killed December 6, 1901, three months and twelve days after injection. The autopsy revealed a multilocular abscess at the seat of injection 10 by 20 cm. in size, the pockets of which were filled with a dirty, yellow-gray fluid, in which much solid necrotic material was held in suspension. A small tumor about 2 cm. in diameter was found about 2 cm. below the injection tumor, which it resembled in all respects. The lymph gland in front of seat of injection, directly back of shoulder, was enlarged and converted into an abscess, which closely resembled the abscess at the seat of injection. No other lesions found. Three guinea pigs were inoculated with material from the abscess at the seat of injection; one was killed on February 5, 1902, and found to be affected with generalized tuberculosis, and the other two died, respectively, February 22 and March 5, 1902, affected with generalized tuberculosis.

Record of heifer No. 204.—On September 24, 1901, injected subcutaneously with 10 c. c. of tubercle culture. The culture was isolated from human tuberculous lesions and was strongly virulent for guinea pigs. The injection was followed by the formation of a local abscess, which reached its maximum in about two months and then slowly decreased in size. On April 6, 1903, a little more than one and one-half years after the injection, the heifer was killed. On autopsy no lesions were found except an abscess at the seat of injection 5 by 3 by 2 cm. in size which contained a thick, creamy pus. The pus showed the presence of a few tubercle bacilli under the microscope. Four guinea pigs were inoculated with a small amount of the pus suspended in sterile water. The guinea pigs were killed on

November 9, 1903, and two were found to be affected with generalized tuberculosis, the other two being in excellent condition and free from lesions of disease.

Record of heifer No. 213.—On September 24, 1901, injected subcutaneously, back of left shoulder, with 10 c. c. of human tubercle culture, which was strongly virulent for guinea pigs. The injection was followed by the formation of an abscess which was punctured on February 4, 1902, four months and eleven days after the injection was made, and about 20 c. c. of soft, necrotic material pressed out. Microscopic examination of the material showed the presence of innumerable tubercle bacilli, isolated and in clumps of from fifty to a hundred. Four guinea pigs were inoculated with small portions of this material; of these one died on April 14, 1902, and three were killed on May 5, 1902. All four were affected with generalized tuberculosis.

On September 19, 1902, the heifer received a subcutaneous injection back of the right shoulder of 10 c. c. of virulent bovine tubercle culture. This was followed by the formation of a local tumor, at first firm and sensitive and afterwards nonsensitive and fluctuating. On November 2, 1903, the heifer was killed. The autopsy showed the presence of a small abscess, which contained a thick, creamy pus, at the seat of the first or human tubercle culture injection. Two guinea pigs were inoculated with material from this abscess. At the seat of the second or bovine tubercle culture injection a much larger abscess was found, which contained a soft necrotic material. The lymph gland near this lesion was greatly enlarged and contained two foci of tuberculosis, each about 10 mm. in diameter. Two guinea pigs were inoculated with material from the lesions in the lymph gland. No other lesions found.

There is no reason for supposing that the infection which persisted at the seat of the earlier injection was in any way associated with the later injection. One of the two guinea pigs inoculated with material from the abscess due to the human tubercle culture injection died on January 11, 1904, and the other was killed on February 3, 1904. Both were affected with generalized tuberculosis. The two guinea pigs inoculated with material from the lymph gland near the seat of injection with bovine tubercle culture died, respectively, December 27, 1903, and January 25, 1904. Both were affected with generalized tuberculosis.

Record of heifer No. 245.—On February 7, 1902, inoculated subcutaneously with tuberculous tissue from a guinea pig which had succumbed to an inoculation with tuberculous material from a human lung. The inoculation was followed by the formation of a local tumor, which reached its maximum in about six weeks and then gradually decreased in size. The heifer was killed on September 29, 1902, seven months and twenty-two days after inoculation. The autopsy showed no lesions except an abscess about 2 cm. in diameter at the seat of the inoculation. The abscess contained a soft, necrotic material, with a portion of which two guinea pigs were inoculated. On December 11, 1902, the guinea pigs were affected with generalized tuberculosis.

Record of heifer No. 243.—On April 12, 1902, inoculated subcutaneously with tuberculous tissue from a human mesenteric gland. The inoculation was followed by the formation of a local tumor, which reached its maximum in about two months and then gradually deceased in size. The heifer was killed on October 1, 1902, five months and nineteen days after the inoculation. The autopsy showed no lesions except a patch of induration at the seat of inoculation, in the center of which an abscess about 3 cm. in diameter was located. The abscess contained a soft, necrotic material, with a portion of which a guinea pig was injected. The guinea pig was killed December 11, 1902, and was found to be affected with generalized tuberculosis.

Record of heifer No. 249.—On April 14, 1902, inoculated subcutaneously with tuberculous tissue from a monkey which had succumbed to an inoculation with human tuberculosis. The inoculation was followed by the formation of a local tumor, which reached its maximum in about one month and then gradually decreased in size. The heifer was killed September 29, 1902, five and one-half months after inoculation. The autopsy showed no lesions except a small induration at the seat of inoculation, in the center of which an abscess 10 to 12 mm. in diameter was located. The abscess contained a soft, necrotic material, with a portion of which a guinea pig was inoculated. The guinea pig was killed December 11, 1902, and was found to be affected with generalized tuberculosis.

Record of steer No. 228.—On January 4, 1902, inoculated subcutaneously with tuberculous tissue from a monkey which had succumbed to an inoculation with human tuberculosis. The inoculation was followed by the formation of a tumor, which reached its maximum size in about two months, then broke and discharged a thick, turbid, gelatinous material. Microscopic examination of the discharged material failed to show tubercle bacilli, but two guinea pigs inoculated with it on February 18, 1902, died, respectively, on June 13 and July 20, 1902, affected with generalized tuberculosis. The abscess continued to discharge until early in March, then closed and remained as a firm nodular tumor in the subcutaneous tissue. The steer was killed on September 24, 1902, eight months and twenty days after inoculation, and on autopsy showed no lesions, except an induration 7 cm. in diameter at seat of inoculation, in the center of which a small abscess was located. The abscess contained a soft, necrotic material, with a portion of which four guinea pigs were inoculated.

The guinea pigs were killed on November 26, 1902, and were found to be affected with generalized tuberculosis.

Record of cow No. 211.—Cow No. 211 received five intravenous injections of moderately virulent human tubercle culture, 10 c. c. on each of the following dates: July 19, August 6, August 20, September 20, and October 7, 1902. She was exposed for about six months—from January 27, 1903, to August 6, 1903—to tuberculosis in a stable which contained, among other cattle, three cows affected with advanced tuberculosis. On August 6, 1903, the cow was killed. On postmortem examination her entire lung was found to be sprinkled with minute, pearl-gray nodules 1 mm. diameter. The mediastinal glands contained a number of circular, red patches, but no definite tuberculous lesions. The costal and pulmonary pleura were thickened and roughened. Microscopic examination of stained sections of the tubercles in the lung showed the presence of tubercle bacilli, isolated and in clumps. Two guinea pigs were inoculated with fragments of the red spots in the mediastinal glands. They were killed two and one-half months later and were found to be in good condition and free from disease. Two guinea pigs were inoculated with minute nodules from the lung. One died after two months and the other was killed after two and one-half months. Both were affected with generalized tuberculosis. The lung lesions in this cow can not be reasonably attributed to any other cause than the intravenous injections of moderately virulent human tubercle culture. The time which elapsed between the last injection received by the cow and the inoculation of the two guinea pigs which contracted generalized tuberculosis is one day less than ten months. The lesions found in the cow were probably healing and would have disappeared in the course of time. How much longer they would have retained their infectious character remains an unsolved question.

A summary of the results obtained is given in the table on the following page.

TABLE VII.—*Statement showing persistence of tubercle bacilli in cattle after injection.*

Animal.	Material injected into cattle.	Date and kind of injection.	Number of guinea pigs inoculated.	Date of guinea pigs' inoculation.	Result of guinea-pig inoculation.	Time elapsed between cattle and guinea-pig inoculations.
Heifer No. 216	Mixture of human tubercle and streptococcus cultures.	Subcutaneous; August 24, 1901.	3	December 6, 1901	Generalized tuberculosis	3 months 12 days.
Heifer No. 204	Human tubercle culture	Subcutaneous; September 24, 1901.	4	April 6, 1903	Two generalized tuberculosis and two unaffected.	1 year 6 months 12 days.
Heifer No. 213	do	do	4	February 4, 1902	Generalized tuberculosis	4 months 11 days.
Do	Bovine tubercle culture	Subcutaneous; September 19, 1902.	4	November 2, 1903	do	2 years 1 month 18 days;[a] 1 year 1 month 13 days.
Heifer No. 245	Human tuberculosis; tissue from a guinea pig.	Subcutaneous; February 7, 1902.	2	September 29, 1902	do	7 months 22 days.
Heifer No. 243	Human tuberculous mesenteric gland.	Subcutaneous; April 12, 1902.	1	October 1, 1902	do	5 months 19 days.
Heifer No. 249	Human tuberculosis; tissue from monkey.	Subcutaneous; April 14, 1902.	1	September 29, 1902	do	5 months 15 days.
Steer No. 228	do	Subcutaneous; January 4, 1902.	4	September 24, 1902	do	8 months 20 days.
Cow No. 211	Human tubercle culture	Intravenous; 5 injections, from July 19 to October 7, 1902.	2	August 6, 1903	do	9 months 29 days.

[a] Two of the four guinea pigs were inoculated with material from the lesion due to the injection of human tubercle culture on September 24, 1901, and two with material from the lesion due to the injection of bovine tubercle culture on September 19, 1902. The lesions produced by the two injections were in no perceptible manner connected with each other.

DISCUSSION OF THE EXPERIMENTS.

The cattle from which the material was taken for these investigations were not specially inoculated to study the persistence in their tissues of tubercle bacilli of insufficient virulence to affect them with a progressive tuberculosis. Otherwise the animals would have been kept alive and material from their lesions tested at regular intervals by microscopic examinations and guinea-pig inoculations, and such tests would have been continued until it was clearly shown that tubercle bacilli were no longer present or had lost their infectiousness. The investigations show that tubercle bacilli remain in the tissues and retain their infectious character for a long time, but give no data from which the maximum time of such persistence can be determined. The fact that the bacilli were present and infectious for guinea pigs in every lesion tested, whatever the age, seems to imply that for practical purposes it is not unreasonable to assume that tubercle bacilli, once they get into the tissues, are in there to stay, whether they produce a progressive disease or not. Whether any multiplication of the tubercle bacilli takes place in the tissues of animals in which no progressive disease occurs can not be answered, but multiplication is not necessary for the persistence of the germs. Neither is it necessary for the production of an affection which is stationary or in the progress of healing, as is shown by the following case:

AN EXPERIMENT WITH STERILE TUBERCLE CULTURE.

Sheep No. 46, in excellent health, tested with tuberculin without reaction. On July 19, 1902, received an injection into the right jugular vein of 10 c. c. of dead bovine tubercle culture, the culture having been killed by exposing it to a temperature of 98° C. for one hour. Several guinea pigs were injected with portions of the culture as a check on its sterility. The guinea pigs were killed three and one-half months later and found to be in excellent condition and free from all lesions of disease. The sheep was killed on January 6, 1903, not quite six months after the injection was made, and the following lesions found: Sprinkled over the entire surface of the lung, immediately under the pleura, are innumerable minute tubercles, all of about the same size—1 mm. diameter—excepting where several have coalesced. Similar tubercles are found, on section, to be sprinkled throughout the lung tissue. (In every respect these tubercles were like the tubercles found in the lung of cow No. 211.) The minute tubercles were somewhat more numerous in the caudal portion of the principal lobes of the lung than in other portions of the organ. The distance between the tubercles was from one-half to 1 cm., excepting in a few regions, in which areas of the lung about 3 cm. square were thickly beset with very minute, necrotic points. The general appearance was that of miliary tuberculosis of the lung. The individual tubercles had the appearance of opaque, pearl-gray points, surrounded by a ring of translucent, glistening tissue. Mediastinal and bronchial glands free from disease, with the exception of a few reddened patches. One of the throat glands was entirely necrotic. All other organs normal. Three guinea pigs injected with material from the lung and three with material from the throat gland were killed eight months later and found to be in excellent condition and free from all lesions of disease. Sections made of the nodules in the lung of the sheep and examined under the microscope showed the presence of innumerable tubercle bacilli.

In other words, the dead germs not only caused extensive disease, but were retained in countless numbers in the tissues for nearly six months. The sheep at no time following the injection showed symptoms of disease, and remained in excellent condition, and was fat and apparently in perfect health when it was killed.

THE PERSISTENCE OF AN INNOCUOUS TUBERCLE CULTURE.

Finally, we wish to add the records of three cattle which received intravenous injections of a tubercle culture of such low virulence that it was innocuous for guinea pigs on subcutaneous injection. These animals were killed 9 months and 18 days, 10 months and 16 days, and 10 months and 28 days, respectively, after receiving the last injection. The lungs of the three animals were thickly sprinkled with minute tubercles, with which tubercle germs were associated. The records are as follows:

Record of cow No. 219.—In excellent condition. Tested with tuberculin without reaction. From April 13, 1903, to January 16, 1904, received eight intravenous injections of attenuated tubercle culture. This culture was originally isolated from human tuberculous lesions, and during its cultivation on artificial media in the Bureau of Animal Industry laboratories had lost its infectiousness for guinea pigs. The injections were gradually increased from 5 to 50 c. c. As a result of the injections the cow had a slight cough, but otherwise remained visibly unaffected. She was killed on November 4, 1904, 9 months and 18 days after she received the last injection. The post-mortem examination revealed numerous minute tubercles in the lung, of a pearl-gray color, and numerous small dark areas in the liver, which, on section, were found to be very much softer than the adjacent tissue. Microscopic examination of stained sections of the tubercles in the lung showed tubercle bacilli, isolated and in clumps composed of numerous germs.

Record of cow No. 280.—In excellent condition. Tested with tuberculin without reaction. From November 18, 1903, to January 27, 1904, received six intravenous injections of attenuated tubercle culture, of the same kind used for cow No. 219, in doses which were gradually increased from 1 to 25 c. c. As a result of the injections she suffered in no visible way, excepting a slight temporary trembling after two or three injections. Killed on December 19, 1904, 10 months and 16 days after the last injection. On autopsy the lung was found to be sprinkled with innumerable minute, translucent nodules, up to 1 mm. diameter, many of which had pearl-gray centers. Microscopic examination of these nodules revealed tubercle bacilli, isolated and in clumps consisting of numerous germs.

Record of heifer No. 305.—The record of this animal is in all respects similar to that of cow No. 280, with the exception that she received eight intravenous injections of attenuated tubercle culture and lost somewhat in condition after the third and fourth. At the time she was killed, December 14, 1904, she was fat and in excellent general condition. The autopsy revealed lung lesions, with tubercle bacilli of the same kind as found in cow No. 280. The time which elapsed between the last injection of tubercle culture and the day on which she was killed is 10 months and 28 days.

The bacilli injected into the last three animals, and cow No. 211 and sheep No. 46, represent three distinct degrees of virulence as this is measured by guinea-pig inoculations. One animal received absolutely dead bacilli; three received bacilli no longer infectious for guinea pigs, and one received bacilli strongly infectious for guinea pigs, but capable of producing only strictly localized lesions in cattle. On post-mortem examination practically identical pathological conditions were found in the five animals, which demonstrates conclusively that cultures of tubercle bacilli, no matter how low their infectiousness is, can not be injected into the blood vessels of animals without causing extensive pathological changes in one or more organs, and, what is more significant, lesions in which the injected bacilli are incorporated and remain long periods of time (in the case of cow No. 211, without losing their infectiousness). Hence, the intravenous or subcutaneous injection of live tubercle cultures of any degree of virulence into animals, whatever else it may do, certainly makes the tissues of the animals a repository for the injected tubercle bacilli during an indefinite period of time, the duration of which is, estimated from the evidence we have, very long in relation to the length of the animal's life.

Methods of immunization which require that one disease be substituted for another or that a disease of minor severity be induced to prevent a more serious affection, can not be approved unless the danger and suffering, on one hand, are comparatively insignificant and the protection gained, on the other hand, is very valuable. The process which protects must stand in proportion to the immunity gained in the ratio of a very small to a very large figure, and even then, if the method is applied to animals and not to man, it is necessary to answer one more question, namely, Is its practice a source of danger to man? Until this latter question can be answered in the negative no system of treatment for the benefit of cattle or other live stock is justifiable or reasonable.

We are of the opinion that immunization of animals to tuberculosis through the injection of live tubercle bacilli into their bodies, with our present imperfect knowledge regarding the comparative susceptibility of man to the affection, should not be practiced otherwise than for experimental purposes in connection with investigations which may in time contribute to our general knowledge of tuberculosis and its treatment and its prevention.

THE NUMBER OF THE TUBERCLE BACILLI FOUND IN THE ANIMALS.

One more table is added to show the length of time tubercle bacilli, of insufficient virulence to cause a progressive or permanent disease in cattle, persisted in the tissues of cattle in sufficient numbers to be detected by a microscopic examination. This table is desirable because, while the injection of guinea pigs with tuberculous tissue is a very delicate test as to whether or not tubercle bacilli are present, it is not nearly so valuable as a microscopic examination when it is desired to ascertain in what numbers the bacilli are present.

TABLE VIII.—*Statement showing number of tubercle bacilli present in cattle at varying periods after injection.*

Animal.	Number and kind of injection.	Material injected.	Time elapsed since last injection.	Result of microscopic examination.
Heifer No. 204	1, subcutaneous	Human tubercle culture.	1 year 6 months 12 days.	A few tubercle bacilli present.
Heifer No. 213	do	do	4 months 11 days	Innumerable tubercle bacilli, isolated and in clumps of from 50 to 100.
Steer No. 228	do	Human tuberculosis, monkey tissue.	2 months	Microscopic examination of discharge from local tumor failed to show tubercle germs.
Cow No. 211	5, intravenous	Human tubercle culture.	9 months 29 days	Numerous tubercle bacilli present.
Sheep No. 46	1, intravenous	Sterile bovine tubercle culture.	5 months 17 days	Innumerable tubercle bacilli present.
Cow No. 219	8, intravenous	Human tubercle culture, innocuous for guinea pigs.	9 months 18 days	Numerous tubercle germs in stained sections of nodules in the lung, isolated and in clumps.
Cow No. 280	6, intravenous	do	10 months 16 days.	Same as preceding (cow No. 219).
Heifer No. 305	8, intravenous	do	10 months 28 days.	Same as preceding.

The general microscopic appearance of the nodules found in the lungs of cattle Nos. 211, 219, 280, and 305 and sheep No. 46 was substantially the same. Each nodule consisted of a small mass of homogeneous material containing innumerable tubercle bacilli. This was inclosed in a connective tissue wall and surrounded by a ring of cell-infiltrated lung tissue.

Guinea pigs were inoculated with material from the lesions found in the lungs of cattle Nos. 219, 280, and 305.[a] Insufficient time has passed since these inoculations were made for the occurrence of the observable disease. The guinea pigs will probably throw no additional light on the lesions in the three cattle, because the attenuated tubercle bacillus injected into the cattle was so weak in its pathogenic character that, on subcutaneous injection, it rarely produced tuberculosis in guinea pigs.

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[a] The guinea pigs inoculated with material from the lungs of cows Nos. 219, 280, and 305, have just been killed this date, April 17, 1905, and examined post-mortem. Six guinea pigs were inoculated with material from each cow, a total of 18 guinea pigs. The time which elapsed between the inoculation and the death of the guinea pigs is four months or longer, amply sufficient for disease to develop. With one exception the 18 guinea pigs were found to be in excellent condition and free from lesions of disease. The one exception, one of the six guinea pigs inoculated with material from cow No. 280, was affected with tuberculosis of the lung, liver, and spleen. The character of the lesions indicated that the animal would have lived for some time if it had not been killed. The results from the guinea pig inoculations bear out the statements made about the mildly infectious nature of the tubercle bacillus injected into the cows, and show that the innocuousness under ordinary circumstances of tubercle bacilli for cattle does not mean that the bacilli, after injection into cattle, are rapidly eliminated or destroyed. In fact, the contrary is shown, namely, that tubercle bacilli of the lowest degree of infectiousness have the power to resist disintegration for long periods of time in the tissues of animals.

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